Lignin dynamics in arable soils as determined by ^{13}C natural abundance

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Zusammenfassung

Lignin ist das zweithäufigste natürliche Polymer nach den Polysacchariden Cellulose und Hemicellulose. Es ist ein wesentlicher Bestandteil von pflanzlichen Zellwänden. Diese werden durch Lignin stabilisiert und vor mikrobiellem Abbau geschützt. Aufgrund des Vorkommens in Pflanzenmaterial wird Lignin auch in den Abbauprodukten, d.h. in der organischen Substanz in Böden und Sedimenten gefunden. Wegen seiner komplexen phenolischen Struktur und der Tatsache, dass nur spezialisierte Pilze Lignin direkt abbauen können, war Lignin lange Zeit als refraktäres Makromolekül bekannt, das selektiv angereichert wird und zur stabilisierten organischen Bodensubstanz beiträgt. Diese ältere Lehrmeinung wurde in den letzten Jahren sehr in Frage gestellt. Neuere Studien konnten zeigen, dass der Ligninumsatz im Boden schneller erfolgte als der Umsatz der gesamten organischen Bodensubstanz. Umsatzzeiten von <1 bis 38 Jahren wurden für Lignin vorgeschlagen.

Das Hauptziel der vorliegenen Arbeit war, den Abbau von Lignin im Boden quantitativ für Langzeitfeldexperimente zu beschreiben, um die vorgeschlagenen Umsatzzeiten zu überprüfen. Weitere wichtige Ziele waren die Auswirkung von landwirtschaftlichen Bewirtschaftungsmethoden, wie (i) mineralische Düngung und (ii) Einarbeitung von Ernterückständen auf den Abbau zu testen, sowie Hinweise für die Stabilisierung von Lignin in bestimmten Bodenfraktionen zu sammeln.

Um den Abbau und die Stabilisierung von Lignin quantitativ über mehrere Dekaden im Boden zu verfolgen, wurde die Markierung von organischer Substanz durch natürliche Kohlenstoff-isotopenhäufigkeit in 18 und 36 Jahre dauernden Dauerfeldversuchen verwendet. Diese Markierungsmethode basiert auf den unterschiedlichen ^{13}C zu ^{12}C Verhältnissen in Pflanzen mit verschiedenen Photosynthesewegen. Die Umstellung von C_3 (z.B. Weizen) auf C_4 Pflanzen (z.B. Mais) bewirkt die Markierung der organischen Bodensubstanz. Die Methode ist nicht nur für die gesamte organische Substanz sondern auch für einzelne Komponenten wie Lignin geeignet. Die Markierung kann u.a. zum Verfolgen der Abbaudynamik verwendet werden. Lignin wurde aus archivierten Bodenproben durch alkalische Oxidation mit Kupferoxid extrahiert. Die Oxidationsprodukte, Lignin-spezifische Monomere, wurden mittels Gaschromatographie quantifiziert. Die Zusammensetzung der stabilen Kohlenstoffisotopen wurde mittels Isotopen-Verhältnis Massenspektrometrie ermittelt.

Der Abbau von C_3-bürtigem Lignin in organischer Bodensubstanz konnte für die untersuchten Feldexperimente am besten durch doppelt-exponentielle Dynamik beschrieben werden. Dabei hatte schnell abbaubares Lignin eine Umsatzzeit von 3 Jahren, langsam-abbaubares Lignin eine Umsatzzeit von 90 Jahren. Das Ergebnis deutet darauf hin, dass die Umsatzzeiten möglicherweise langsamer sind als in anderen neuen Studien. Die Ergebnisse sollten jedoch nicht überinterpretiert werden, da die Zahl der Messdatenpunkte für Zeitreihen länger als 30 Jahre immer noch sehr gering ist. Mineraldüngung hat in dem 36 Jahre dauernden Feldversuch nicht dazu geführt, dass der Abbau von Lignin langfristig verringert wurde. Unter Feldbedingungen wich das Ergebnis wegen der Vielschichtigkeit von Einflussfaktoren von dem älterer Laborstudien ab, die eine Reduktion des Abbaus unter erhöhter Stickstoffdüngung festgestellt hatten. Die Einarbeitung von Ernterückständen erhöhte erwartungsgemäss die Gesamtkonzentrationen an organischem Bodenkohlenstoff und Lignin. Ein gesteigerter Abbau von ursprünglich im Boden vorliegendem C_3-bürtigem Lignin konnte aber nicht festgestellt werden, woraus sich ableiten lässt, dass dieses unberührt gebliebene Lignin im Boden bereits stabilisiert wurde. Tatsächlich konnte in beiden Langzeitfeldexperimenten nach 18 oder 36 Jahren jeweils noch mindestens ca. 60 bzw. 40 % des markierten Lignins von Beginn des Experiments wiedergefunden werden. Eine Fraktionierung des Bodens deutete darauf hin, dass besonders die gröbere partikuläre organische Substanz und die Schluff-Fraktion Lignin stabilisieren könnten. Im Zusammenhang mit Ligninstabilisierung wurde bereits in früheren Studien auf die Schluff-Fraktion hingewiesen. Der mögliche Einfluss von Mineralen auf den Schutz vor Abbau wurde auch bei der partikulären organischen Substanz festgestellt, die von einer mineralischen Kruste umgeben war, ganz ähnlich einem frühen Stadium der Aggregatbildung.

Aufgrund der Ergebnisse kann geschlussfolgert werden, dass Lignin tatsächlich innerhalb von einigen Jahrzehnten abgebaut wird. Es gibt jedoch Hinweise, dass ein Teil des Lignins im Boden stabilisiert wird, wahrscheinlich durch Minerale, welche die Organische Substanz physikalisch vor dem Angriff von Abbauenzymen schützen. Die Dynamik des Ligninabbaus konnte durch die untersuchten Bewirtschaftungsbedingungen nicht verändert werden. Unter Feldbedingungen im Zeitraum von Jahrzehnten könnten komplexe Rückkopplungsmechanismen jedoch stärkere Auswirkungen haben als spezifische mechanistische Effekte, die in Laborexperimenten für Zeiträume von Monaten bis wenigen Jahren festgestellt wurden.

Weiterführende Forschungsrichtungen wären zum einen substanz-spezifische Studien zu Lignin in Langzeitfeldversuchen (auch andere Landnutzungen, z.B. Grasland oder Wald), bei denen bereits Auswirkungen der Düngung auf die gesamte organische Bodensubstanz festgestellt wurden. Es gilt noch herauszufinden, ob eine Verringerung des Abbaus der gesamten organischen Bodensubstanz tatsächlich mit einem geringeren Ligninabbau in Zusammenhang steht. Eine weitere vorgeschlagene Richtung wäre die Erforschung der Mechanismen für die Stabilisierung von Lignin im Boden über mehrere Jahrzehnte. Dies könnte über kontrollierte Markierungsexperimente mit Aggregaten erfolgen.

Summary

Lignin is the second most abundant polymer in nature after the polysaccharides cellulose and hemicellulose. It is a main component in plant cell walls, where it has stabilizing and protective functions. Because of its abundance in plant material, lignin can also be found in the decomposition product organic matter in soils and sediments. Due to its complex phenolic structure and the fact that only specialized fungi can decompose it, lignin has long been referred to as a very stable macromolecule that contributes to stabilized organic matter. This older belief of lignin as a refractory molecule, which is selectively preserved, has been much challenged recently. Lignin has been found to turn over faster than bulk organic matter and turnover times of <1 to 38 years have been proposed.

The main objective of this thesis was to quantitatively describe lignin decomposition in long-term field experiments in order to validate the proposed turnover times. Other important objectives were to test the effect of arable management practices (i) mineral fertilization or (ii) biomass incorporation on decomposition and to provide evidence on soil fractions that retain lignin.

To track the decomposition and retention of lignin quantitatively over decades, labeling with natural carbon isotopic abundance was taken advantage of in an 18-year and a 36-year continuous maize field experiment. Labeling is based on the different ^{13}C to ^{12}C ratios in plants with different photosynthetic pathways. Conversion from C_3 vegetation (e.g. wheat) to C_4 vegetation (e.g. maize) induces labeling of the soil organic matter. Lignin was extracted from archived soil samples by alkaline cupric oxide oxidation, which is an established method for soils and sediments. The oxidation products, lignin-specific monomers, were quantified using gas chromatography and the stable carbon isotopic composition was analyzed by isotope ratio mass spectrometry.

Decomposition of C_3-derived lignin in soil organic matter could best be described by double-exponential decay dynamics for the studied experiments. The fast pool had a turnover time of 3 years, the slow pool of 90 years. The results suggest that turnover might not be as fast as proposed recently from other experiments. Interpretation is however still limited because data for time periods of longer than 30 years is scarce. Mineral fertilization did not retard lignin decomposition in the long-term in the studied 36-year experiment. Due to the complexity of the agro-ecosystem the results differed from earlier controlled lab studies, proposing that fertilization might have contradicting effects in the field. Biomass incorporation naturally increased the total amount of SOC and lignin in the soils, but had no priming effect on initial C_3-derived lignin, suggesting that these lignin moieties might have been stabilized in soil. In fact, in both long-term field experiments after 18 or 36 years still at least 60 or 40 % of the initial C_3-derived lignin was detectable. A fractionation study for the 18-year experiment indicated lignin might have been retained in the coarse particulate organic matter fraction or in the free silt fraction. The silt fraction had been proposed earlier as a possible fraction for lignin stabilization, suggesting mineral-organic interactions. The retention in free particulate organic matter could also be related to interaction with minerals, because organic matter was protected with a mineral crust, as in early stages of aggregation.

From this study it can be concluded that lignin decomposes within decades in soil. However, it seems that a portion of the lignin is to a certain extent stabilized in soil, most likely through a form of protection by soil minerals. The decomposition dynamics could not be influenced by management, suggesting that in the long-term (decades) complex ecosystem feedbacks might outweigh distinct priming effects found in short-term studies (months to years).

Proposed research perspectives are the compound-specific investigation of long-term field experiments (also other land uses, e.g. grassland or forest), where fertilization effects on soil organic carbon decomposition had been shown previously in order to find out if lignin is involved in the slow decomposition. Another direction of further study could be to explore the mechanisms of lignin retention over decades, which could be assessed e.g. in controlled labeling experiments with aggregates.

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Part A Synopsis

1. Introduction

1.1 Lignin: from plant cell walls to soil organic carbon

It is still not known how some of the initial lignin input from plant litter might be stabilized in soil, but it is quite certain that the intrinsic structure of lignin might not be the only cause.

Lignin compounds are aromatic polymers in the cell walls of vascular plants (Sarkanen & Ludwig, 1971; recent detailed chemical definition reviewed by Monties & Fukushima, 2001). Because lignin concentrations in plant material constitute up to 25 mass-% of the plant dry weight (Zeikus, 1981; Buranov & Mazza, 2008), lignin is the second most abundant polymer in nature after the polysaccharides cellulose and hemicellulose, with which it is closely associated (Derenne & Largeau, 2001; Buranov & Mazza, 2008). Lignin strengthens plant cell walls, decreases water permeation, and protects cellulose from biotic damage by microorganisms (Zeikus, 1981; Monties & Fukushima, 2001; Kögel-Knabner, 2002). Since the lignin macromolecule consists of irregularly ordered, non-repeating aromatic subunits that are linked by diverse chemical bonds (Figure 1), lignin is relatively resistant to rapid biological decomposition in comparison to carbohydrates, lipids or proteins (Derenne & Largeau, 2001; Gleixner et al., 2001). Only white rot fungi (basidiomycetes) seem to be able to selectively decompose lignin by extracellular enzymes, otherwise decomposition occurs co-metabolically (Swift et al., 1979; Kirk & Farrell, 1987; Hatakka, 2001; Martínez et al., 2005). In the process of plant litter (leaves, stems, roots) decomposition, lignin becomes part of organic matter in soil or sediments (Derenne & Largeau, 2001; Kögel-Knabner, 2002; Horwath, 2007).

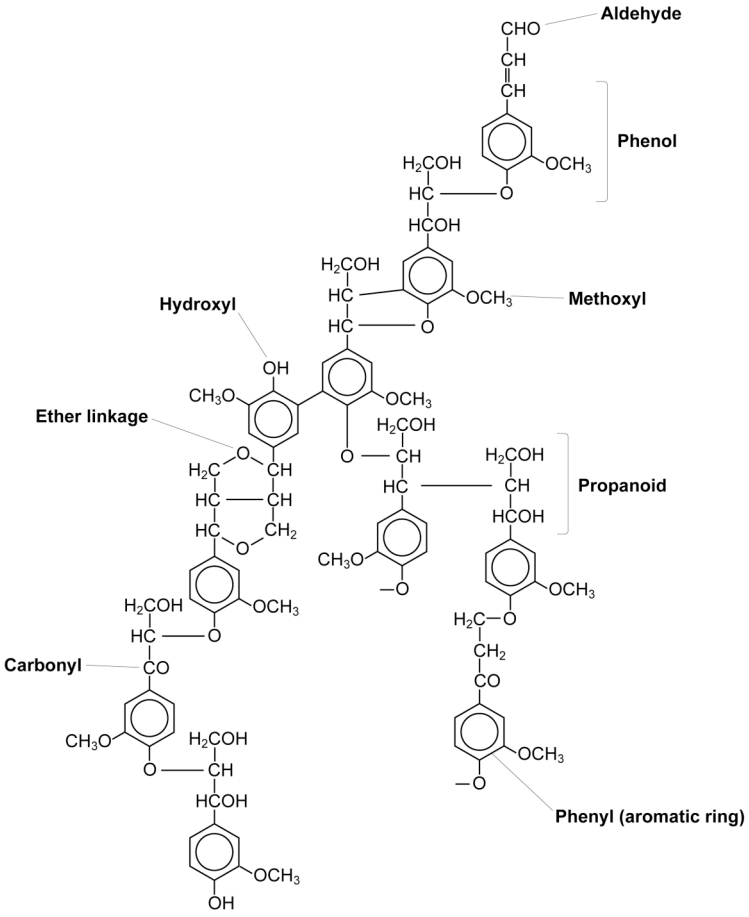


Figure 1 Generalized lignin structure as presented by Paul and Clark (1996). A generalized lignin structure was also offered by Monties and Fukushima (2001). An early structure of lignin that is often cited, was proposed by Adler (1977). For recent lignin model structures, including a 3D-structure, see online material of Boerjan et al. (2003). A previous 3D model of lignin was proposed by Faulon and Hatcher (1994).

Decomposition (synonyms: decay, degradation) transforms plant litter by (i) physical fragmentation (e.g. through macrofauna), (ii) leaching of water-soluble compounds, (iii) chemical transformation (oxidation or condensation) and (iv) biological metabolism, including extracellular enzymes (Berg & McClaugherty, 2008). Eventually, this important ecological process leads - via various stages of intermediate decomposition products (soil organic matter eq. humus) - to complete mineralization, which recycles important plant nutrients like nitrogen, phosphorus, potassium and releases CO_2 to the atmosphere (Sanderman & Amundson, 2005). Melillo et al. (1989) described decomposition processes in ecosystems as a "continuum beginning with fresh plant litter and leading to the formation of refractory soil organic matter". Soil organic matter (SOM) thus consists of partially decomposed, hardly recognizable residues of plants, microorganisms and soil fauna (e.g. Horwath, 2007). SOM is subjected to further decomposition in the soil (Sanderman & Amundson, 2005). The processes and mechanisms involved in SOM dynamics (decomposition and stabilization) are a key research area in the field of soil science.

Lignin has a special role in soil and sedimentary organic matter research due to its (i) unique origin from vascular plants which makes lignin a perfect plant biomarker (e.g. Hedges & Mann, 1979; Goñi et al., 2003) and especially due to its (ii) intrinsic resistance to decomposition (Derenne & Largeau, 2001) which makes it a potentially long-lived moiety of soil organic carbon. The theory of lignin as a refractory compound of organic carbon (e.g. Zeikus, 1981) was supported by findings from litter decay studies where initial lignin concentrations of litter were negatively correlated with decay rate constants (Melillo et al., 1982). In a 2-year incubation experiment with ^{14}C-labelled lignin, Martin el al. (1980) found that decomposition was rapid in the first 6 months and then leveled off. Because the residual lignin was recovered in the humic acid fraction, Martin el al. (1980) concluded that "major portions of the lignin carbon are incorporated into the more resistant or aromatic portions of the soil humus". As a consequence from these results, lignin was recognized to be an important control in decomposition. Many quantitative models of soil organic matter dynamics assume that the majority of plant lignin is directly transformed into slow soil organic carbon (Plante & Parton, 2007).

Concentrations of lignin in mineral soil and in sediments are commonly measured after oxidation with cupric oxide (VSC-lignin, Hedges & Ertel, 1982, discussed in section 3.3). Important pioneer studies on lignin in soils were the research on lignin in forest humus layers by Kögel and Bochter (1985) and Kögel (1986) and of lignin in physical soil fractions by Guggenberger et al. (1994) and Amelung et al. (1999). In bulk mineral soil, total lignin concentrations range from about 20 mg per kg soil in depleted arable soils to 1.2 g per kg soil in soils heavily fertilized with farmyard manure (data review in Appendix, Table 1; Kiem & Kögel-Knabner, 2003). Lignin concentrations (VSC) in soil organic carbon range from approximately 2 to 64 g per kg SOC (Appendix, Table 1). When considering that this macromolecule is thought to be relatively resistant to decomposition, higher concentrations of lignin in soil organic carbon might be expected.

Why should one compound be preferentially retained in soil? In fact, the older belief of lignin being an especially recalcitrant component of soil organic carbon was much challenged in the last years by publications suggesting that lignin turnover was faster than SOC turnover (Kiem & Kögel-Knabner, 2003; Heim & Schmidt, 2007a). Another important result was that lignin turnover times did not exceed 38 years in arable soil or grassland (Gleixner et al., 2001; Dignac et al., 2005; Bahri et al., 2006; Heim & Schmidt, 2007a; 2007b, reviewed by Amelung et al., 2008; discussed in section 4.1). On the basis of data from the 9-year field experiment analysed by Dignac et al. (2005), Rasse et al. (2006) developed a two-pool model of lignin decomposition in mineral soil. Lignin in the fast pool had a turnover time of less than 1 year and only 8 mass-% of the initial lignin from plant residues reached the slow pool where it was somewhat protected from rapid decomposition (turnover time 20 y). From the accumulated recent results Marschner et al. (2008) concluded that there is "obviously no inherent recalcitrance of the lignin molecule itself". Instead, lignin might be protected (on the time scale of decades) rather by interaction with soil minerals (Rasse et al., 2006; Heim & Schmidt, 2007b). Long-term field studies of several decades (covering the proposed turnover times) with a relatively high sample resolution could be used to corroborate the new findings on lignin.

1.2 Tracking decomposition of soil organic carbon and its components

Natural stable carbon isotope labeling of soil organic carbon is one of the techniques that can be used to quantitatively track decomposition processes on a compound-specific level.

Decomposition of soil organic matter (SOM) can be quantitatively assessed in laboratory or field experiments by (i) measurement of SOM-derived CO_2 efflux from soils (basal respiration, e.g. Kuzyakov, 2006), (ii) radiocarbon methods based on labeling of SOM by ^{14}C (e.g. Trumbore, 1993; Amundson et al., 1998) and by (iii) labeling with stable carbon isotopes (^{12}C, ^{13}C), based on free air CO_2 enrichment (FACE, e.g. Moran & Jastrow, 2009) or the difference in carbon isotope fractionation between C_3 and C_4 plants (vegetation conversion, Balesdent et al., 1987; Balesdent & Mariotti, 1996) which averages 14 ‰ (O'Leary, 1981).

Stable carbon isotope studies based on vegetation conversion have the advantage that they can be implemented as field experiments without costly installations since labeling is achieved by conversion to C_4 vegetation on a C_3 soil or vice versa (Balesdent et al., 1987). One prerequisite is however the continuous input of the respective labeled biomass, which is only fulfilled in few field experiments because it requires continuous cropping of C_4 or C_3 vegetation respectively (as opposed to arable rotation that might mix C_4 or C_3 vegetation).

How does the stable carbon isotope label work? The carbon atom in carbon dioxide (CO_2) in the atmosphere consists to about 98.9 % of the lighter stable carbon isotope ^{12}C. In comparison, the heavier ^{13}C has a natural abundance of only 1.1 % ($^{14}C < 10^{-9}$ %). Because of their specific metabolism, plants with a C_4 photosynthetic pathway (e.g. maize, miscanthus) discriminate less against the heavier ^{13}C at CO_2 fixation during photosynthesis, than plants with a C_3 photosynthetic pathway (e.g. barley, wheat, beets). Less discrimination subsequently translates into more uptake, which is mirrored by higher ^{13}C concentrations in C_4 plant material in comparison to C_3 plant material (O'Leary, 1981; Meier-Augenstein, 1999). These differences in the natural abundances of the stable carbon isotopes are detectable in all stages of the plant life and are introduced to soil organic matter by plant litter.

Because the differences in ^{13}C concentrations are very small at the natural abundances level, they are expressed in delta notation as the per mil (‰) difference between the sample and an international standard (Vienna PeeDee Belemnite, VPDB; Boutton, 1996; Amelung et al., 2008). Equation 1 shows the calculation of $\delta^{13}C$ values (Balesdent & Mariotti, 1996; Glaser, 2005).

$$\delta^{13}C\,(\text{per mil}) = \frac{[R_{sample} - R_{standard}]}{R_{standard}} \cdot 1000 \qquad \text{(Equation 1)}$$

where $R_{sample} = \frac{^{13}C}{^{12}C}$ and $R_{standard\ (VPDB)} = 0.0112372$ (e.g. Glaser, 2005).

$\delta^{13}C$ values can be determined for both bulk organic carbon in soil and plants, and also for specific compounds, e.g. for lignin monomers (“compound-specific stable isotope analysis”, CSIA, Amelung et al., 2008). The data can be used in a mixing model to assess the fraction of labeled carbon (= C_4-derived carbon in a conversion from C_3 to C_4 vegetation) as shown in Equation 2 (Balesdent & Mariotti, 1996; Bernoux et al., 1998; Dignac et al., 2005; Heim & Schmidt, 2007a).

$$F_{labeled\,C} = \frac{\delta^{13}C_{soil\,labeled} - \delta^{13}C_{soil\,reference}}{\delta^{13}C_{plant\,label} - \delta^{13}C_{plant\,reference}} \qquad \text{(Equation 2)}$$

$F_{labeled\ C}$ is the fraction of labeled carbon, $\delta^{13}C_{soil\ labeled}$ is the delta value for soil under conversion, $\delta^{13}C_{soil\ reference}$ the delta value for the soil in year 0 of the experiment or in a parallel experiment without conversion (assumption: no change in carbon stocks), finally the lower term is the difference between delta values of the plant material that labels the soil, $\delta^{13}C_{plant\ label}$, and the plant material that was the input prior to the conversion.

Labeling with stable isotopes can be applied to bulk (=total) SOC or to specific compounds and is primarily used to determine turnover or mean residence times (Glaser, 2005; Amelung et al., 2008). Turnover or mean residence times (MRT) are defined as the inverse of the decay rate constant, in Equation 3 solved for mono-exponential decay (Balesdent et al., 1990; Heim & Schmidt, 2007a).

$$MRT = \frac{1}{k} = \frac{-t}{(\ln(1 - F_{labeled\,C}))} \qquad \text{(Equation 3)}$$

where k is the decay rate constant, t is the time since conversion started, $F_{labeled\ C}$ is the fraction of labeled carbon from Equation 2.

A decay rate constant of e.g. 0.03 yr^{-1} would mean that annually 3 mass-% of the total lignin concentration in the pool was replaced by new lignin. The resulting turnover time of 1/k ≈ 33 years for mono-exponential decay thus indicates when about 37 % of the initial concentration is left (e^{-1} ≈ 0.368). Since in this example each year only 3 % of the total mass is replaced, it would still take about 154 years until only 1 mass-% of the initial lignin concentration was left (calculated using Equation 4).

$$t = \frac{\ln(f)}{-k} \qquad \text{(Equation 4)}$$

where t is the time after which a certain fraction f of the initial concentration remains in the system with the decay rate constant k. Figure 2 visualizes the example.

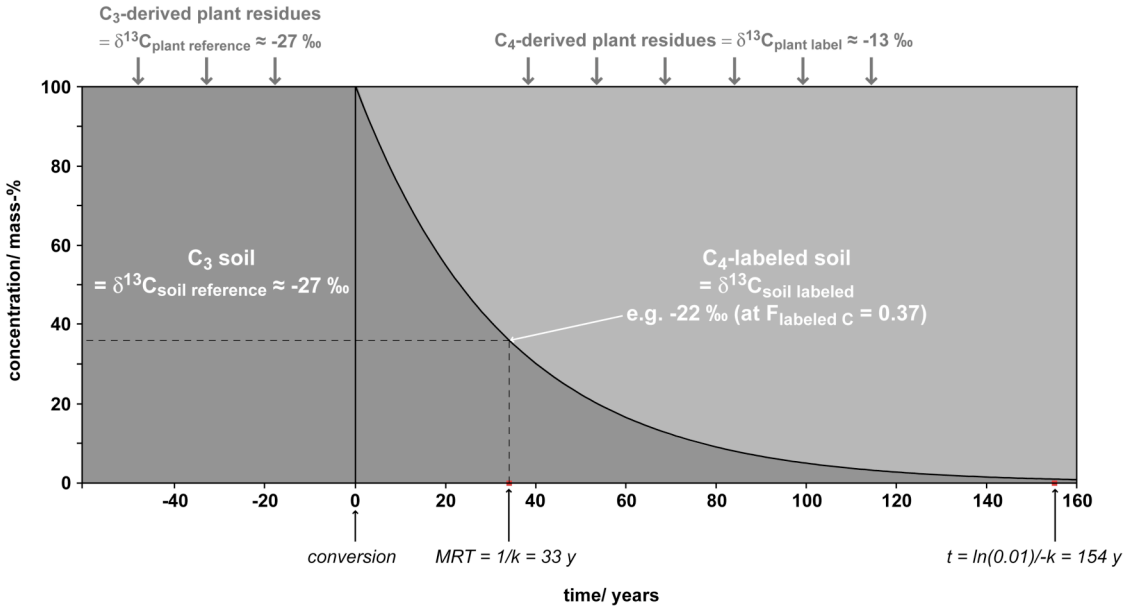


Figure 2 Replacement of C_3-derived soil organic carbon by C_4-derived soil organic carbon after vegetation conversion (example for mono-exponential decay with the decay rate constant k = 0.03).

As shown in Figure 2, labeling with stable isotopes can actually also be applied to quantitatively trace decomposition of bulk SOC and even compound-specifically for lignin. In this case the concentration decline is directly measured over time. This is possible, because lignin can only be synthesized by plants. Lignin cannot be recycled by microorganisms in soil, and thus concentrations of labeled lignin can only decrease. For this quantitative approach, samples of the start of the experiment (year 0) are required and a relatively high sample frequency is needed. When applied to long-term field experiments, the approach can be used to directly evaluate the decay kinetics of lignin, e.g. to validate one or two-pool models.

1.3 Can arable soil management practices be applied to control decomposition?

A large proportion of the mitigation potential of agriculture arises from soil carbon sequestration, which has strong synergies with sustainable agriculture and generally reduces vulnerability to climate change (IPCC, 2007).

Article 3.4 of the Kyoto Protocol (UNFCCC, 1997; Appendix) implies the task for soil science to find out about the potential of soils to store and release carbon (Smith et al., 2000; Scholes & Noble, 2001). Soils are the largest reservoir of actively cycling carbon in terrestrial ecosystems. To a depth of 1 m soils store about 75 % (≈ 1500 Pg carbon, 1 Pg = 10^9 t) of the total organic carbon in terrestrial ecosystems, the remaining 25 % is stored in living plant biomass (≈ 550 Pg carbon; Prentice et al., 2001). The role of soil organic matter in the global carbon cycle (Houghton, 2005) has triggered immense research (Janzen, 2004). Understanding how carbon cycles in soil is crucial, because through decomposition or stabilization of organic matter, soils become sources or sinks for greenhouse gases and thus might impact the climate (Trumbore & Czimczik, 2008).

Biological mitigation of CO_2 in terrestrial ecosystems (e.g. forests, agricultural land) can be achieved by (i) conservation of existing carbon pools, (ii) sequestration, i.e. storage of additional organic carbon, by increasing the size of carbon pools, and (iii) substitution with sustainably produced biological products, e.g. wood for energy intensive construction products and biomass for fossil fuels (IPCC, 2001). However, when studying the potential of soils to mitigate CO_2, it is important to keep in mind that e.g. carbon sequestration in soil is only a limited, short-term response to rising atmospheric CO_2 concentrations (Scholes and Noble, 2001). “Although natural sinks can potentially slow the rate of increase in atmospheric CO_2 there is no natural savior waiting to assimilate all the anthropogenic CO_2 in the coming century” (Falkowski et al., 2000). Still, conservation and sequestration of carbon may allow time for other options to be further developed and implemented (IPCC, 2001).

Anthropogenic disturbance (e.g. land-use change, fertilization, biomass burning) is known to control decomposition of organic matter (Bird et al., 2001), besides the general factors moisture, temperature, microbial activity and substrate quality (i.e. chemical composition of the decomposing material: nitrogen, carbohydrate, lignin concentration; e.g. Plante & Parton, 2007). One important anthropogenic factor for the release of CO_2 is land-use change. Conversion from forest or grassland to arable land commonly leads to a decline in soil organic carbon concentrations. This is because disturbing soils (e.g. by plowing) releases organic matter that was previously protected by aggregates and aerates the upper soil horizon with oxygen, thus increasing microbial activity and decomposition of organic material (Paustian et al., 2000). A second reason for the decline in soil organic carbon in arable soil is the export of products from the field at harvest (Janzen, 2004). How can land be managed to retain more carbon and thereby mitigate the increasing concentration of atmospheric carbon dioxide? With this question, the research on decomposition of soil organic matter can well be set into a management context. This is especially valid for already intensively managed agro-ecosystems. Increasing depleted organic carbon stocks of arable soils not only has the potential to sequester carbon, but also has numerous benefits for soil structural stability, nutrient availability and soil organisms (Lal, 2004).

A number of management options were proposed for carbon sequestration in arable soils, some examples are reduced or zero-tillage, incorporation of organic amendments (animal manure, sewage sludge cereal straw), increased production efficiency and alternative long-term land use of surplus arable land (Paustian et al., 1997; Smith et al., 1997; Bruce et al., 1999; Schlesinger, 1999; Paustian et al., 2000; Smith et al., 2000; Freibauer et al., 2004; Lal, 2004; Smith et al., 2007; Smith et al., 2008; Govaerts et al., 2009). An additional factor on changes in soil organic carbon is nitrogen enrichment. There is an ongoing discussion about the effects of nitrogen fertilization (either atmospheric deposition or intended fertilization) on soil organic matter in arable, grassland and forest ecosystems (Khan et al., 2007; Reay et al., 2008). A direct link to lignin is drawn (Heimann & Reichstein, 2008) because there is evidence that lignin decomposition might be retarded by added nitrogen (Keyser et al., 1978; Fog, 1988; Berg & Matzner, 1997; Carreiro et al., 2000; Kuzyakov et al., 2000; Craine et al., 2007). These negative nitrogen-effects, i.e. retardation

of decomposition, were for example explained by (i) reduction of competitive ability of basidiomycetes (decomposers of lignocellulose) and (ii) induced repression of enzyme synthesis (Fog, 1988). Keyser et al. (1978) found that ligninase was produced only when available nitrogen was low. Fog (1988) suggested that "lignin may be considered as a matrix, in which more easily decomposable substrates (polysaccharides) are embedded. If nitrogen hampers lignin decomposition, then the matrix material cannot be broken up, and most of the matrix material remains unavailable. The overall process of decomposition will be flagging." Negative or no effects were found to occur both in the lab and in the field, mostly in experiments lasting months or years, but have not been shown yet in long-term field experiments.

The effects of nitrogen on decomposition can be categorized in the broad field of priming effects, that are defined by Kuzyakov et al. (2000) as "strong short-term changes in the turnover of soil organic matter caused by comparatively moderate treatments of the soil, i.e. input of organic or mineral fertilizer, exudation of organic substances by roots, mechanical treatment, drying and rewetting". Figure 3 visualizes the concept of positive and negative priming effects, here adapted for declining concentrations of e.g. organic carbon or its component lignin in soil.

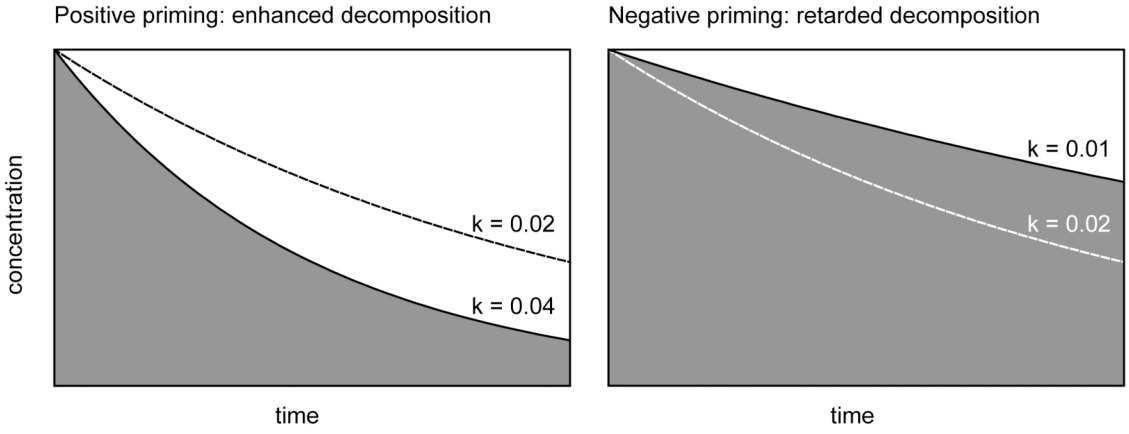


Figure 3 Priming effects in an example for mono-exponential decay.

As suggested from the findings by Keyser et al. (1978), Fog (1988) and Carreiro (2000), lignin decomposition in soil might be affected by priming when soil is fertilized with nitrogen. The validity of the results in field experiments is however not clear yet. Additionally, not only nitrogen fertilization but also the input of fresh organic matter might affect decomposition dynamics. Increased biomass input might stimulate the decomposition of older organic matter in soil, because it might stimulate the microbial activity (Wang & Bakken, 1997). Since lignin is degraded co-metabolically, the stimulation of microbial activity might enhance lignin decomposition.

The relevance of priming effects in the context of arable management practices and carbon retention can be focused in the following question: What happens to older organic matter when the above stated management practices for carbon sequestration are applied?

2. Objectives

This thesis can contribute to the quantitative description of lignin decomposition in long-term (decades) arable soil field experiments. Contribution can also be made to the evaluation of potential factors on lignin decomposition such as fertilization or biomass incorporation, that have so far only been studied in short-term (months to years) experiments. In addition, this thesis aims at contributing to the current discussion on stabilization mechanisms of soil organic matter, by relating physical soil fractions to potential stabilization mechanisms of lignin. The thesis thus seeks to provide new insights in the area of basic soil organic matter research as well as in applied research, because the field experiments are based on relevant arable soil management practices that might be important for storing organic matter in soils.

The research is based on unique long-term field experiments in temperate arable soils that provide a stable carbon isotope label (^{13}C natural abundance) in the organic carbon of soil organic matter. By applying the established methods for lignin analysis in soil (Hedges & Ertel, 1982; Goñi & Montgomery, 2000) and compound-specific stable-isotope analysis (Glaser, 2005; Amelung et al., 2008) to these ^{13}C labeled long-term field experiments, the thesis can give information on the decay or stability of lignin in arable soils for decadal time periods under field conditions. It can thus help to complement or verify results of short-term or lab studies, so not to draw misleading conclusions, e.g. on effects of arable management practices such as fertilization, for long-term field conditions.

These are the questions that can be answered with this study:

1. Are lignin decay dynamics of long-term field experiments in accordance with recent turnover time calculations?
Hypothesis: A time series covering several decades with high temporal resolution (soil samples every 3 to 6 years) might allow to test if the mono-exponential decay pattern, assumed in lignin turnover time calculations (Heim & Schmidt, 2007a), actually fits to lignin decay dynamics or should be replaced by double-exponential decay models as proposed by Lobe et al. (2002) and Rasse et al. (2006).

2. Does mineral fertilization retard lignin decomposition?
Hypothesis: Lignin decomposition might be reduced as in a negative priming effect (Kuzyakov et al., 2000) due to retardation in enzymatic activity of microbes as suggested by Keyser et al. (1978), Fog (1988) and Carreiro et al. (2000).

3. Does incorporation of additional biomass enhance lignin decomposition?
Hypothesis: Lignin decomposition might be enhanced as in a positive priming effect (Kuzyakov et al., 2000) due to stimulation of microbial activity and subsequent co-metabolic decay (Wang & Bakken, 1997).

4. In which soil fractions is lignin stabilized?
Hypothesis: Because lignin originates from plant cell walls, in soil, lignin might be most abundant and stabilized in (i) free particulate organic matter that comprises almost original or only slightly decomposed plant material or in (ii) occluded particulate organic matter that is physically protected in soil aggregates (Christensen, 1996a; Golchin et al., 1998; von Lützow et al., 2006; 2007).

3. Material and Methods

This chapter gives an overview of the materials and methods that were used to answer the research questions. The focus of the thesis was on soils from agricultural land-use, specifically arable (i.e. plowed) soils. This focus helped to specifically look at two defined arable soil management practices as factors on lignin decay, namely mineral fertilization and biomass incorporation (return of harvest residues to the soil, e.g. maize stover). These practices are common treatments for arable soils and are also applied in field experiments. Through collaborations with Bent T. Christensen (Research Centre Foulum, University of Aarhus, Denmark) and Paola Gioacchini (Institute of Agricultural Chemistry, University of Bologna, Italy) we had the opportunity to access archived soil samples of two long-term field experiments. The decadal time scale of these experiments added time as a third factor on lignin decay, which was important for the study because only long durations of experiments could show if certain management practices might affect organic matter in general and lignin as a component in particular. These long durations in combination with a relatively high resolution of soil samplings were also essential for testing different patterns (kinetics) of decay dynamics.

3.1 Long-term field experiments

Manuscripts I and III of this thesis are based on archived soil samples from the continuous silage maize experiment initiated in 1988 by Bent T. Christensen in Askov, Denmark. In short, the part of the experiment we used, consists of two treatments, (i) a small input treatment of maize organic carbon only from stubbles and below-ground biomass (roots and rhizodeposits) and (ii) a large input treatment with additional organic carbon input of coarsely chopped above-ground maize biomass (stems, leaves, cobs = maize stover) incorporated into the soils in autumn after harvest (800 g dry matter m^{-2} $year^{-1}$; Kristiansen et al., 2005). The amount of maize stover incorporated into the soils approximately matches the amount of maize harvest residues left on the field after grain harvest (Barber, 1979). However, the two treatments cannot be exactly equalized with silage maize cropping (small input treatment) and grain maize cropping (large input treatment) because the stover is cut at the maturity level for silage maize and does not reach the maturity of grain maize because of climatic conditions in Denmark (Kristiansen et al., 2005). As described in manuscript I, the addition of a known amount of above-ground biomass on top of the unknown amount of below-ground biomass (which is very difficult to quantify exactly, Amos & Walters, 2006) is similar to a spike in chemical analysis. Differences in results between the treatments can then be attributed directly to the large input treatment.

Manuscript II of the thesis is based on archived soil samples from the continuous silage maize field experiment initiated in 1966 by Giovanni Toderi in Cadriano, Bologna, Italy. The treatments of the part of the experiment used for this study consisted of (i) a non-fertilized treatment receiving zero mineral fertilization besides ambient atmospheric nitrogen input (10 to 25 kg N ha^{-1} a^{-1}, Holland et al., 2005) and (ii) a fertilized treatment with mineral fertilization of 300 kg N ha^{-1} a^{-1} and 150 kg P ha^{-1} a^{-1}) applied at sowing and at the four-leaf stadium of maize plants.

Both long-term field experiments were sampled for soil and plant material irregularly every 2 to 6 years. These samples were stored in archives and now sub-samples were taken and analyzed in this study. Plant samples were only archived of aboveground plant material for both experiments unfortunately, thus no samples of root material were available for analysis.

Table 1 provides an overview on the main features of both experiments.

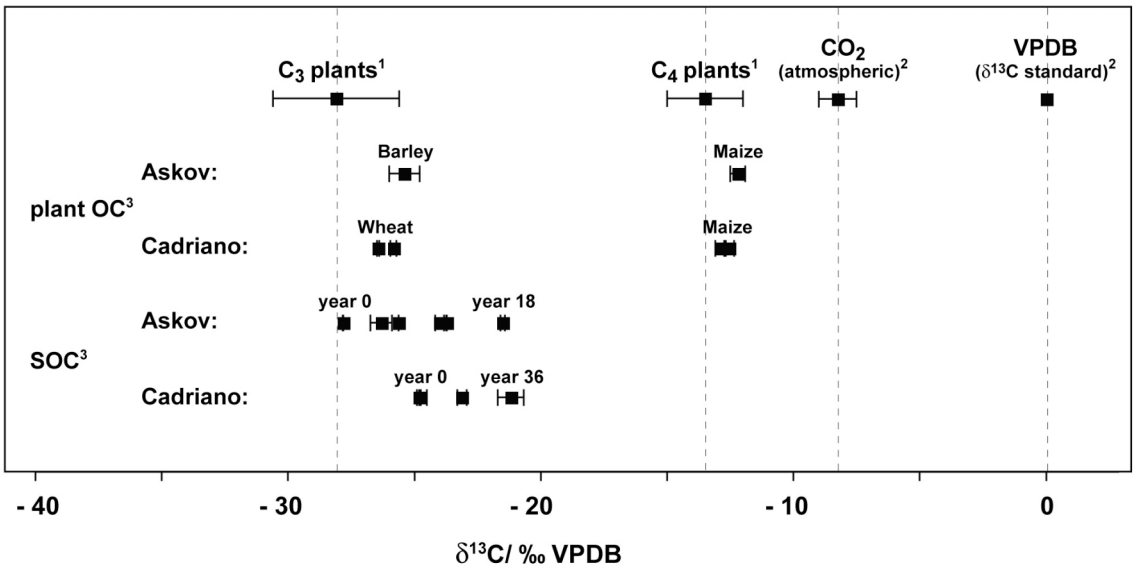
Table 1 Features of the experiments.

	Continuous silage maize experiments		
	Askov	**Lundgaard**	**Cadriano**
Institution (2009)	Aarhus University, Faculty of Agricultural Sciences		University of Bologna, Department of Agricultural Sciences and Technology
Location	55°28'N, 09°07'E		44°32'N, 11°23'56E
Mean ann. temperature	7.7°C		11°C
Mean ann. precipitation	862 mm		650 mm
year started	1988		1966
Experiment type	semi-field experiments in cylinders (diameter 0.7 m, depth 0.5 m)		field experiment, plots
Field replicates	1-2 (homogenized)		2 (separate, except 2002)
Crops prior start	C_3-plants (barley, beets)		C_3-plants (wheat, alfalfa, beets)
Crops after start	maize (C_4)		maize (C_4)
Labeling	natural ^{13}C abundance		natural ^{13}C abundance
Return of maize stover	800 g dry matter m^{-2} yr^{-1} in high input treatment		none (silage maize harvest)
Fertilization/ kg ha^{-1} a^{-1}	N 170-200, P 35-40, K 190		N 300, P 150
Planting	early May		early April
Harvest	mid-October		end of September
Sampling time	Spring, prior to planting		Fall, after harvest
Sampling depth	0-20 cm		0-25 cm (until 1992); 0-40 cm (after 1992)
Sampling years for soil (years after start)	1988 (0), 1991 (3), 1994 (6), 1998 (10), 2003 (15), 2006 (18)		1973 (7), 1980 (14), 1985 (19), 1997 (31), 2002 (36)
Soil type (USDA)	Alfisol (Typic Hapludalf)	Inseptisol (Orchrept)	Typic Udochrept
Soil texture /mass-%			
Sand (50-2000 µm)	64.6	86.8	56
Silt (2-50 µm)	21.2	7.7	16
Clay (<2 µm)	14.1	5.5	28
pH (H_2O)	6.4	7.6	6.9
SOC/ mg g^{-1} soil	25.6 ± 0.5 (1988)	11.0 ± 0.3 (1988)	7.6 ± 0.3 (1973)
C/N	12.9 ± 0.4 (1988)	10.2 ± 0.0 (1988)	7.1 ± 0.4 (1973)
$\delta^{13}C$/ ‰ V-PDB	-27.8 ± 0.0 (1988)	-26.3 ± 0.4 (1988)	-24.7 ± 0.2 (1973, wheat plot)
References	(Christensen, 1997; Kristiansen et al., 2005; Hofmann et al., 2009a)		(Gioacchini et al., 2007; Hofmann et al., 2009b)

3.2 Labeling soil organic carbon by ^{13}C natural abundance

The unique trait of the studied field experiments is that they include a natural stable carbon isotope label of soil organic carbon (Glaser, 2005; Amelung et al., 2008) due to conversion from C_3- to continuous C_4-vegetation (also see section 1.2). Figure 4 compares $\delta^{13}C$ values from the literature (O'Leary, 1981; Hoefs, 2009) and from the studied field experiments.

Depending on (i) the amounts of maize stover returned to the field after harvest and (ii) the experiment's duration, the shift in $\delta^{13}C$ values towards a C_4 signature in soil might be more or less distinct (Flessa et al., 2000). Soil organic carbon in the Cadriano experiment shows a smaller shift despite the longer duration due to the small biomass input. All aboveground plant input was removed from the field when maize biomass was harvested as silage thus the C_4 signature is less pronounced than it would have been in a high input treatment.



[1] O'Leary (1981)
[2] Hoefs (2009)
[3] Data tables in Appendix, background data from Hofmann et al. (2009a, 2009b)

Figure 4 Overview of $\delta^{13}C$ values from the literature in comparison to plant and soil samples of the two field experiments studied (figure adapted from Meier-Augenstein, 1999). Continuous maize cultivation on soil with a C_3-plant signature (year 0) will change the $\delta^{13}C$ values over time to more positive values (year 18, 36). The starting year (year 0) values for the Cadriano experiment are represented by archived soil samples of the parallel continuous wheat plots (Manuscript II).

3.3 Specific aspects of analysis

An overview on the analytical methods applied in this thesis, with references to specific studies and soil science related reviews on the methods, is given in Table 2. This section will not introduce the methods, but rather focus on discussing certain aspects of methods.

Table 2 Overview of the analytical methods applied to study SOC and lignin decay in arable soils (methods are described in detail in the manuscripts).

Target	Method/ equipment	References
Manuscripts I, II, III:		
Carbon and nitrogen concentrations	Elemental analysis (EA)	Tabatabai and Bremner (1991); Nelson and Sommers (1996)
$\delta^{13}C$ of soil and plant organic carbon	EA coupled to an isotope ratio mass spectrometer (EA-IRMS)	Glaser (2005)
Lignin extraction	Alkaline cupric oxide (CuO) oxidation in a microwave digestion system	Hedges and Ertel (1982), Goñi and Montgomery (2000), Heim and Schmidt (2007a)
Quantification of CuO oxidation products (VSC-lignin monomers)	Gas chromatography coupled to a flame ionization detector (GC-FID)	Heim and Schmidt (2007a)
$\delta^{13}C$ of lignin carbon	Compound-specific stable isotope analysis (CSIA) of lignin monomers by gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS)	Goni and Eglinton (1996), Glaser (2005)
Additional in manuscript III:		
Physical soil fractions	Combined particle size and density fractionation of bulk soil without ultrasonic dispersion	Six et al. (1998)
Mineralogical composition	X-ray diffractometry (XRD)	Kahle et al. (2002)
Surface area	5-point BET-N_2 surface area analysis	Kaiser and Guggenberger (2003)
Visualization/ element analysis	Scanning electron microscopy (SEM) coupled to a secondary electron detector (SE) or an energy-dispersive X-ray-detector (EDX)	Goodhew et al. (2001)

Quantification of CuO oxidation products Extraction of lignin from plant material or mineral matrices (soil and sediment) is hindered because lignin is an amorphous polymer that lacks ordered subunits and because lignin is always associated with carbohydrates (Buranov & Mazza, 2008). It is practically impossible to extract lignin in pure form, thus any quantification will be only proximate. In plant sciences lignin is determined gravimetrically as Klason lignin (ash-free residue of H_2SO_4 hydrolysis; standard method for analysis of wood) or as acid detergent lignin (ADL, sequential detergent analysis; frequent method in agronomy; Lin & Dence, 1992; Jung et al., 1999). Because these methods define lignin as a gravimetric residue, they cannot be used to quantify lignin concentrations in soils and sediments that would contain minerals as an additional residue. The common method used to extract lignin from mineral matrices is based on oxidation of the lignin polymer to break it up into defined monomers (Figure 5) and subsequent quantification of eight

lignin-specific oxidation products by gas chromatography (Figure 6; Hedges & Ertel, 1982; Goñi & Montgomery, 2000 as adapted by Heim & Schmidt, 2007a). The concentrations of the eight oxidation products are summed to give VSC-lignin, VSC being the term for the three units of vanillyl, syringyl and cinnamyl phenols. VSC-lignin yields are lower than those of Klason lignin or ADL because for quantification only the eight lignin-specific oxidation products (monomers) are used while actually also other less specific oxidation products such as dimers or trimers are generated (these are not included in quantification). VSC-lignin is thus more specific than the other methods, which is important for the use of lignin as biomarker.

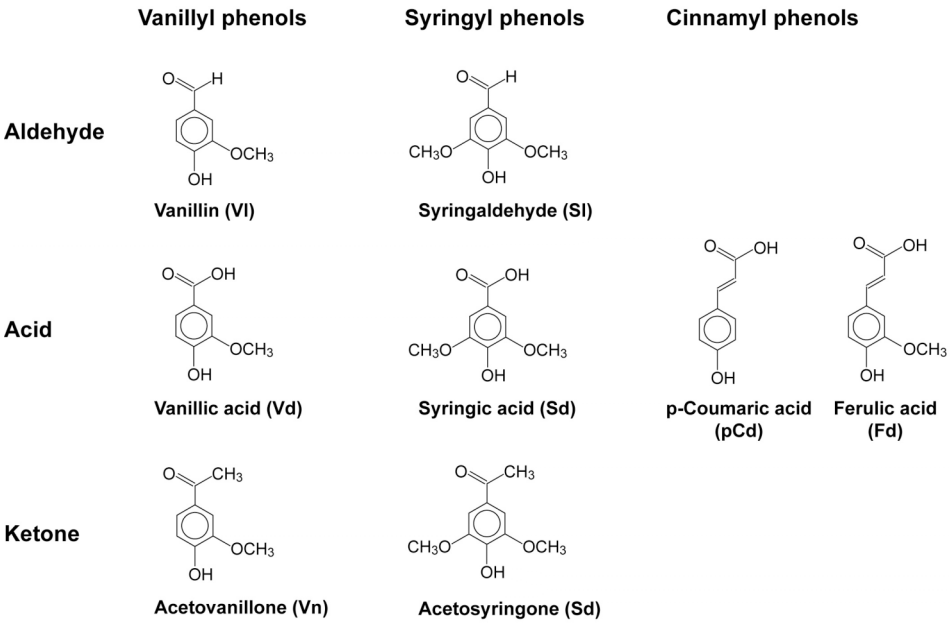


Figure 5 CuO oxidation products specific to lignin (from Heim & Schmidt, 2007a).

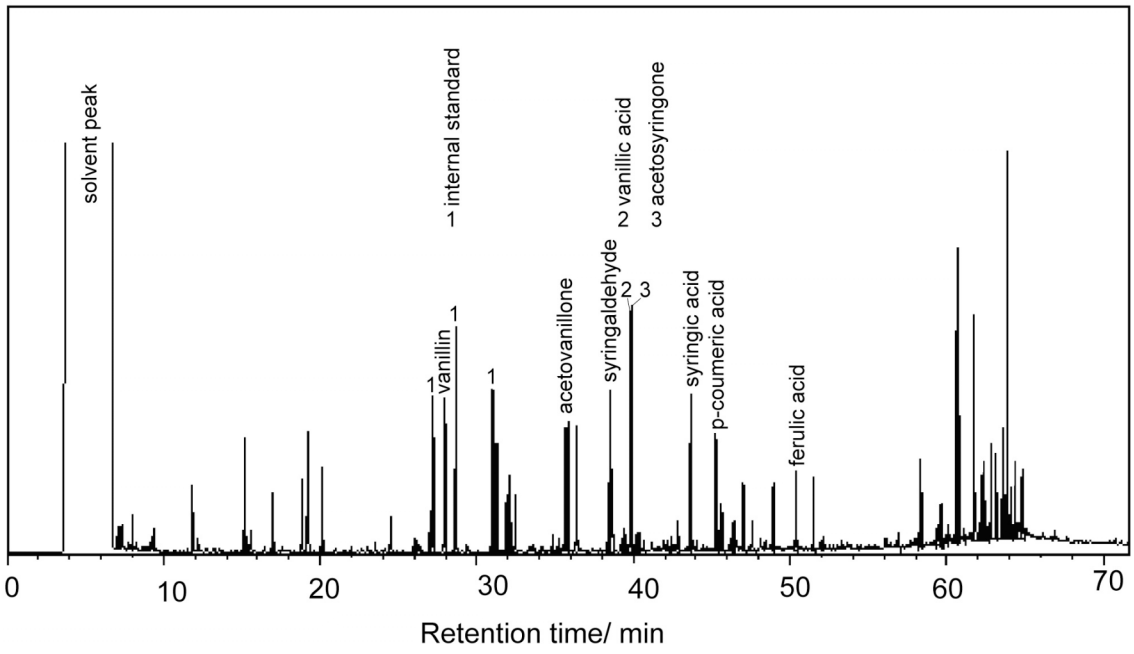


Figure 6 GC-FID measurement of CuO oxidation products from an Askov soil sample.

Correction for $\delta^{13}C$ of the derivatization reagent Gas chromatography of the products of lignin oxidation requires derivatization of the compounds to facilitate volatilization. Silylation is the most widely used derivatization procedure for GC analysis (Knapp, 1979). The reagent for silylation is BSTFA (N,O-bis(trimethylsilyl)triflouroacetamide) in combination with TMCS (trimethylchlorosilane; 99:1 volume mixture). Silylation adds a trimethylsilyl group at the hydroxyl group of the lignin monomer as shown in Figure 7.

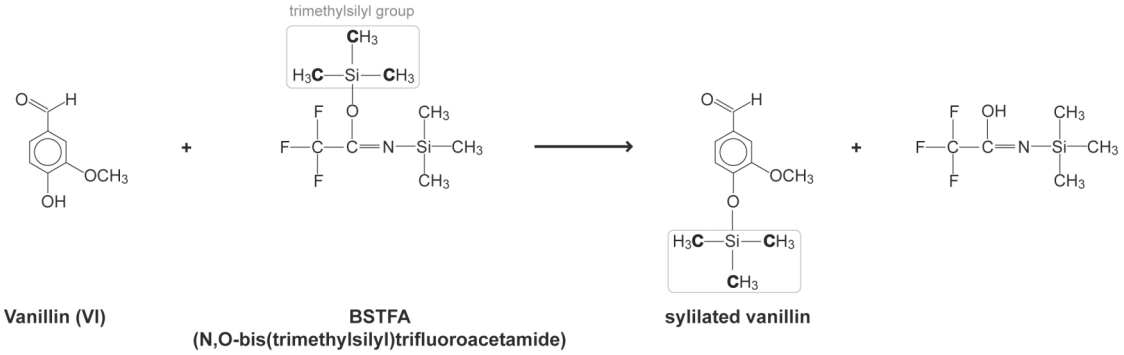


Figure 7 Silylation adds a trimethylsilyl group (marked in the figure) to the CuO oxidation product.

The three carbon atoms that are introduced by the trimethylsilyl group at the derivatization step must be corrected for in the $\delta^{13}C$ values of the specific lignin monomers, because they might shift the $\delta^{13}C$ values of the monomers. The correction in the presented studies was conducted according to the mass balance equation by Dignac et al. (2005) with the exception that BSTFA was measured on-line with the GC-C-IRMS as described in manuscript I in order to obtain a $\delta^{13}C$ value specifically for the GC-C-IRMS application. Figure 8 shows a comparison of corrected $\delta^{13}C$ values with those of underivatized lignin monomer standards measured by elemental analyzer coupled to an isotope ratio mass spectrometer. The corrected $\delta^{13}C$ values were close to those measured off-line, thus verifying the calculations.

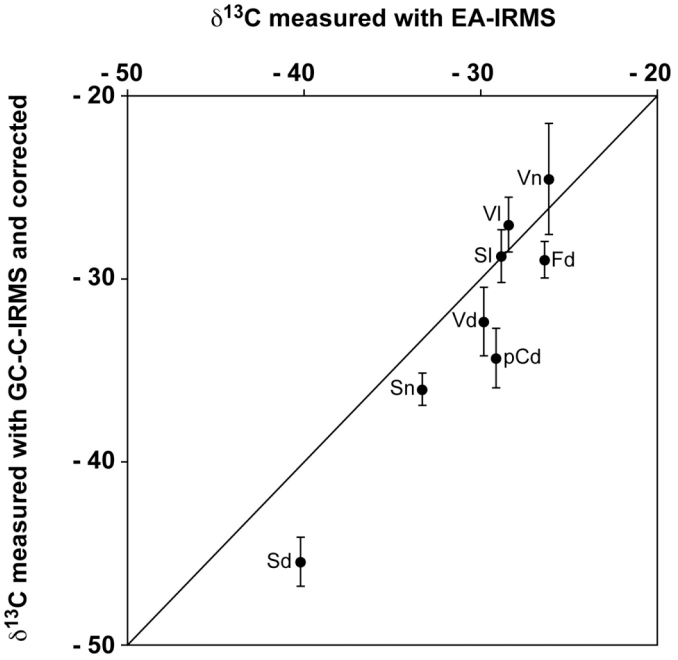


Figure 8 Comparison of $\delta^{13}C$ values of underivatized lignin standards measured with EA-IRMS and trimethylsilylated lignin standards measured with GC-C-IRMS. Error bars represent the standard error for GC-C-IRMS analysis, errors for EA-IRMS are too small to be depicted (*n* = 18).

Lignin carbon (C_{vsc}) In the literature, lignin yields of the CuO oxidation method are commonly presented as the sum of the eight lignin monomers (VSC-lignin). The carbon content in the lignin monomers (8 to 10 C atoms) can be calculated from the molecular formulas of the monomers and is on average 61 mass-% (individual lignin monomers: Vl = 63.15, Vd = 57.14, Vn = 65.05, Sl = 59.34, Sd = 54.55, Sn = 61.22, pCd = 65.85, Fd = 61.85 mass-% carbon). The advantage of calculating the carbon content of VSC-lignin is that lignin carbon then can be directly compared to soil organic carbon. This comparison had been an important aspect in all three manuscripts. The original VSC-lignin results of each manuscript are listed in the appendix.

3.4 Soil fractionation

Manuscript III is based on a soil fractionation with the aim to separate the bulk (=total) soil into components with special traits or functions (von Lützow et al., 2007; von Lützow et al., 2008) that help explain mechanisms or processes of lignin decay or stabilization. Figure 9 presents a scheme of how physical soil fractions might be linked to stabilization mechanisms. The scheme is based on the following assumptions:

(1) Hierarchy of aggregation: Soil mineral particles are organized to form macro- (> 250 μm) and microaggregates (20-250 μm) (Tisdall & Oades, 1982; Christensen, 1996b; Golchin et al., 1998; Jastrow & Miller, 1998) as well as silt- (2-20 μm) or clay-sized (<2 μm) microaggregates (Chenu & Plante, 2006; Virto et al., 2008).

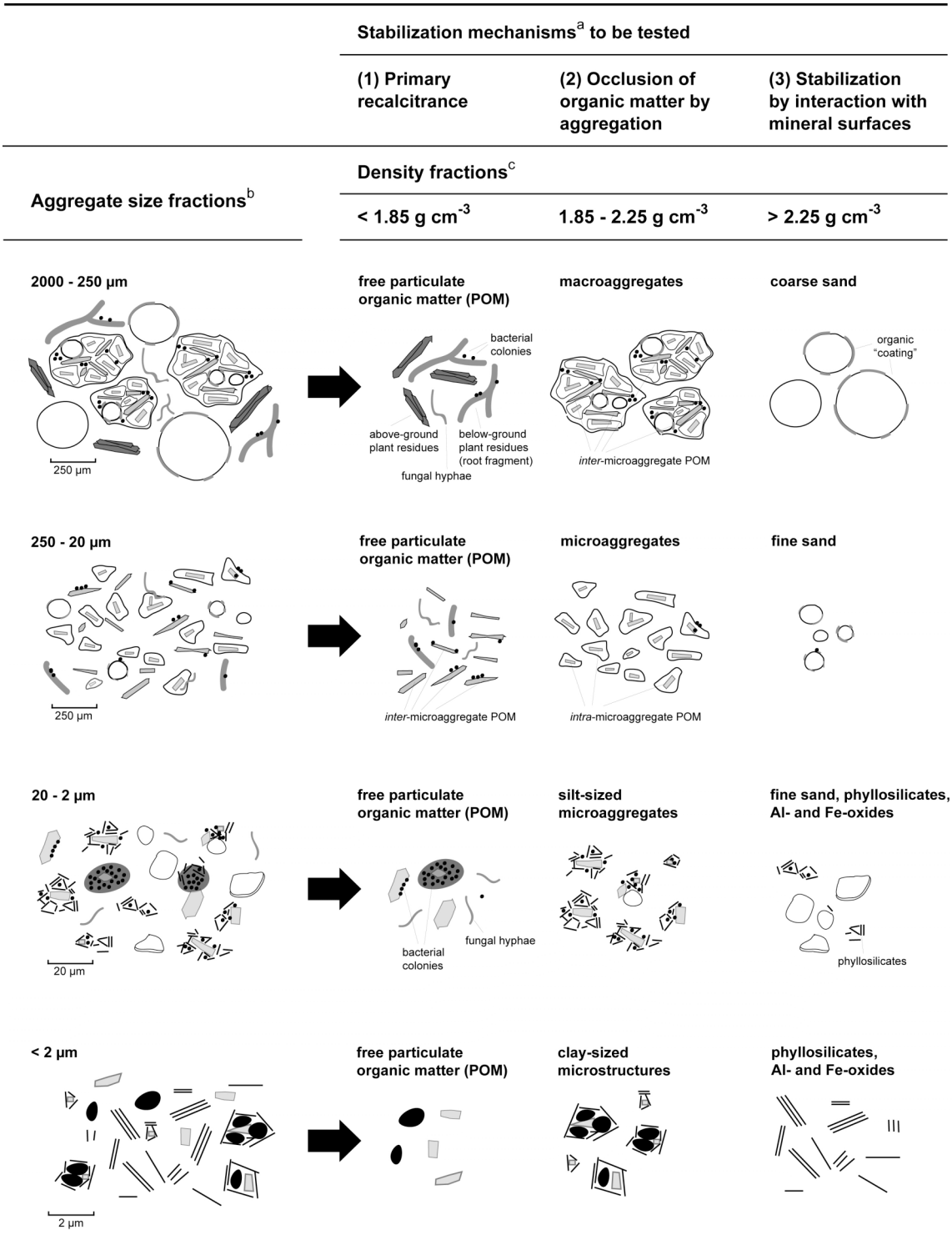
(2) Soil organic matter is a binding agent in the aggregation process (Blanco-Canqui & Lal, 2004; Tisdall & Oades, 1982; Oades, 1984; Six et al., 2000). Organic matter can bind together microaggregates, which creates macroaggregates (inter-microaggregate POM) and can be occluded in even smaller aggregates (intra-microaggregate POM) (Tisdall & Oades, 1982).

(3) Soil fractions are a mixture of mineral and organic components and can vary in density according to the proportions of mineral or organic material (mixing model by Chenu & Plante, 2006), where organic material has a density of 1.4 g cm^{-3} and a mixed mineralogy clay fraction a density of 2.6 g cm^{-3}.

(4) The proposed stabilizaton mechanisms for soil organic matter are (i) primary recalcitrance, (ii) occlusion by aggregation and (iii) stabilization by interaction with mineral surfaces (von Lützow et al., 2006; Amelung et al., 2008; von Lützow et al., 2008).

(5) The state of decomposition and alteration of lignin in soils proceeds with decreasing particle size and increasing density (Guggenberger et al., 1994; Amelung et al., 1999; Sollins et al., 2006).

Based on these assumptions, light density fractions in the scheme (Figure 9) represent free particulate organic matter (fPOM). With smaller particle size fPOM becomes more decomposed. If components of particulate organic matter are retained for a long time in the soil in the light fractions, it can be assumed that they are protected by their chemical structure, which leads to the link of light fractions to primary recalcitrance.
Fractions of medium density are mixtures of minerals and organic matter (Chenu & Plante, 2006) and thus could represent soil aggregates. The different sizes would represent different sizes of aggregates. Lignin that is comprised in this fraction and is still not decomposed after several years might be considered as stabilized by physical separation from the attack of fungal enzymes. Thus the medium fractions might be linked to the stabilization mechanism of occlusion by aggregation.
Finally, heavy fractions consist of soil minerals (quartz, feldspars, clay) and oxides. Organic matter that is associated with these fractions, might be stabilized by the interaction with mineral surfaces.



[a] Lützow et al. (2006)
[b] adapted from Six et al. (1998)
[c] adapted for lignin from Sollins et al. (2006)

Figure 9 Proposal for a fractionation scheme linking physical soil fractions to possible stabilization mechanisms. The different classes of density fractions are not meant as clear-cut categories, they should be seen rather as a continuum from free particulate organic matter to mineral material.

4. Results and discussion

The focus of this results and discussion section will be on C_3-derived lignin, which is at least 18 years (Askov, Lundgaard) or 36 years (Cadriano) old in the studied arable soils, because it originates from the C_3-vegetation prior to the start of the continuous maize experiments. This C_3-derived lignin can only decompose in the soil and not increase during the field experiments because all new lignin introduced to the soil after the start of the experiments derives from C_4-plants (maize) and new C_3 inputs are practically zero. Furthermore, the lignin macromolecule cannot be recycled by microorganisms (as e.g. carbohydrates can, Derrien et al., 2006), which means that the lignin measured in soil can only stem from decaying plant material. When tracking C_3-derived lignin we can therefore directly describe lignin decay in situ.
In the following subsections, results for C_3-derived lignin of the manuscripts are highlighted to specifically (i) discuss lignin decay dynamics (4.1), long-term management factors on lignin decay in the field (4.2, 4.3) and possible mechanisms of lignin stabilization in soils (4.4). Other aspects of the results, like C_4-derived new lignin and SOC (both C_3 and C_4), are part of the results and discussion in the manuscripts. Additionally, complete data sets on all aspects (including plant data and monomers) are provided in the appendix.

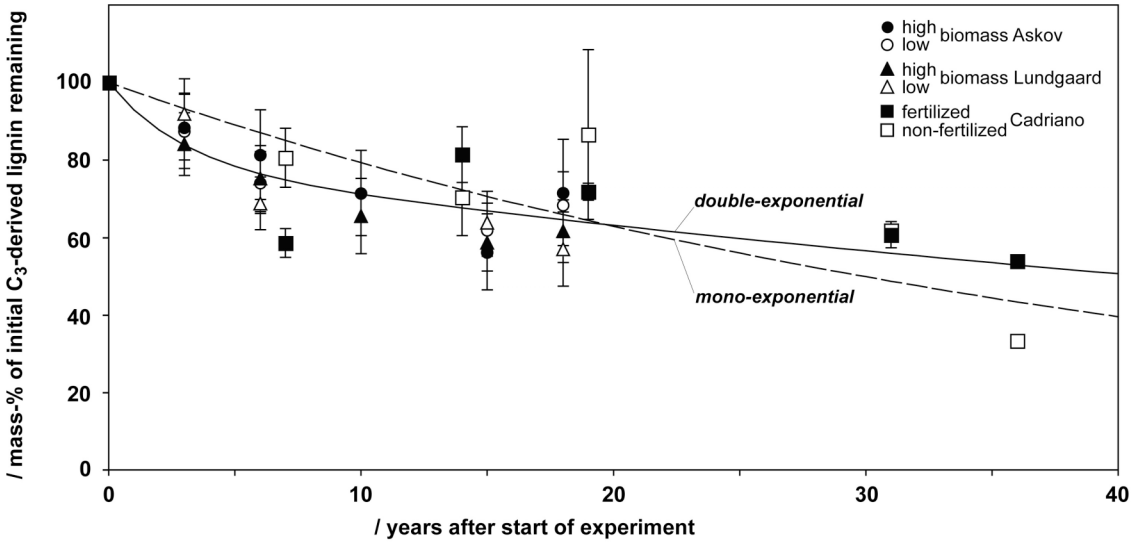
4.1 Lignin decomposition dynamics were slower than expected

One of the main findings for both long-term field experiments was the relative slow mineralization of lignin measured in the soils of the long-term field experiments. After 18 or 36 years at least 60 or 40 mass-% of the initial lignin concentration were still detectable in the studied time series on C_3-derived lignin decomposition in arable soils (Figure 10).

When considering the long-held belief that lignin degrades extremely slowly in soils and contributes to forming humic substances (section 1.1), the above result statement might at a first glance seem to be in alignment. However, the time scales of lignin decay are likely much shorter than those suggested in the older theory. Recent studies proposed turnover times (mean residence times) for lignin of < 1 to 38 years (Dignac et al., 2005; Rasse et al., 2006; Heim & Schmidt, 2007a).

Dignac et al. (2005) calculated lignin turnover times of about 20 years for the Closeaux experimental fields (9 years, maize, C_3 to C_4 conversion). Heim and Schmidt (2007) calculated turnover times of < 9 to 38 years for the Boigneville experimental fields (23 years, maize, C_3 to C_4 conversion). The underlying assumptions for these calculations of turnover times are (i) exponential decay and (ii) constant total concentrations which means steady state of input and decay (Bernoux et al., 1998). The concept of turnover time should be considered carefully because the assumptions might not be met in the experiments. For example, steady state of total concentrations cannot be fulfilled in the Askov continuous maize experiments where it was actually an objective to test biomass input for its increasing effect on total SOC concentration (Kristiansen et al., 2005). For the soils investigated in this study, only the low input treatments in the , Figure 12) and both treatments in Cadriano (Figure 10) would fulfill the steady state assumption. This assumption can be neglected only if the initial concentration at t=0 (A_0) is known. In that case, the initial concentrations can be set to 1 and all concentrations can be calculated as mass-% of initial, like in Figure 10.
The second assumption for calculated turnover times, mono-exponential decay of lignin, has been challenged by the study of Lobe et al. (2002) on lignin decomposition in a subtropical arable soil and by the model of Rasse et al. (2006) based on the lignin data of the Closeaux experimental field (Dignac et al., 2005). Lobe et al. (2002) was the first to propose a two-pool model (double-exponential) to describe lignin decomposition in soil. The study was based on lignin decay due to land-use change from grassland to arable farming (over periods of up to 98 years). In their two-pool model of lignin decay the authors proposed a loss rate of k = 0.15 for a fast (labile) pool constituting ca. 36 mass-% of the initial total lignin concentration and a loss rate of k = 0.0046 for a slow (stable) lignin pool of about 64 mass-%. Because the study of Lobe et al. (2002) does not allow to distinguish between new crop residue lignin inputs and old lignin (no isotopic tracer available) the decay rates might have been underestimated (Rasse et al., 2006).

Rasse et al. (2006) proposed a model with two functionally different lignin pools in soil, (i) lignin in undecomposed plant residues and (ii) lignin partially protected from degradation in soil fractions. In their model, the fast pool comprises about 92 mass-% of the total concentration and has a loss rate of k = 1.88. The slow pool in the model by Rasse et al. (2006) on the other hand has a loss rate of k = 0.052 and comprises the remaining 8 mass-%.



one pool (mono-exponential decay)	two pools (double-exponential decay)
$A_t = A \cdot e^{-kt}$ A_t substrate A remaining at time t e ≈ 2.718 (Euler's number) k decay rate constant = dA/dt t time (yr)	$A_t = A_{fast} \cdot e^{-k_{fast}t} + A_{slow} \cdot e^{-k_{slow}t}$ A_t substrate A remaining at time t e ≈ 2.718 (Euler's number) $A_{fast, slow}$ mass-% of A in fast or slow pool (partitioning between pools) $k_{fast, slow}$ decay rate constant for fast or slow pool
k = 0.023 yr^{-1}	$\mathbf{A_{fast}}$ ≈ 21 mass-% $\mathbf{A_{slow}}$ ≈ 79 mass-% $\mathbf{k_{fast}}$ = 0.341 yr^{-1} $\mathbf{k_{slow}}$ = 0.011 yr^{-1}
Σ **least square differences** = 3545	Σ **least square differences** = 2081
T turnover time 1/k ≈ 44 yr	**T turnover times** $1/k_{fast}$ ≈ 3 yr $1/k_{slow}$ ≈ 90 yr

Figure 10 Decreasing concentrations of C_3-derived lignin over time with decay dynamics (compiled from data presented in manuscripts I and II).

Figure 10 shows the complete data set (combined from two different systems) of measured C_3-lignin concentration decrease, which represents lignin decay in arable soils. Both, mono-exponential as well as double-exponential decay dynamics were fitted to the data set. The best fit was achieved with two pools. One pool would overestimate the decay rate in the longer term (Figure 10). This result would be in accordance with the two pools suggested by Rasse et al. (2006) and Lobe et al. (2002). The result proposes decay rates and mass partitioning closer to that computed by Lobe et al. (2002). The data set is unique because of its long duration with stable isotope labeling, even though there is still too few data points to allow interpretation in the decadal time scale. Eventually we would need to evaluate turnover with field experiments that cover the proposed modeled time scales. Our presently available time series are too short to represent the suggested decay time scales.

How could a relatively slow decomposition of lignin (= slow pool) be explained? Rasse et al. (2006) suggest that the lignin that is slowly degrading might constitute adsorbed lignin fragments or/and

still intact lignin polymers within plant residues that are protected within soil microaggregates. The slow lignin pool might thus be an accumulated residue of plant material that has not yet been decomposed. In manuscript III we propose that the slow lignin pool might be stabilized by one or more of the proposed stabilization mechanisms (i) primary recalcitrance, (ii) physical protection in soil aggregates and (iii) interaction (adsorption) with mineral surfaces (von Lützow et al., 2006). Paragraph 4.4 provides results about the soil fractions in which this old, supposedly stable lignin might reside.

Another important aspect is the question if the results of C_3-derived lignin from C_3 to C_4 conversion studies might be transferred to C_4-derived lignin. This would only work under the assumption of a steady state (input = decay), because large amounts of new lignin entering the system could lead to differing effect such as negative or positive priming (section 4.3).

4.2 Mineral fertilization did not retard lignin decomposition

The second main objective was to find out if lignin decomposition dynamics might be influenced by arable soil management practices such as mineral fertilization or biomass incorporation (section 4.3). As shown in manuscript II, mineral fertilization of the soil did not significantly affect the decay of C_3-labeled lignin over 36 years (Figure 10). This result for a long-term field experiment is in contrast to nitrogen effects on soil microbiology (Fog, 1988; Wang & Bakken, 1997; Magill & Aber, 1998; Henriksen & Breland, 1999; Carreiro et al., 2000) described for lab or semi-field experiments. Some authors reported reduced SOC decay due to reduced lignin decay when nitrogen was fertilized (Keyser et al., 1978; Magill & Aber, 1998; Carreiro et al., 2000; Hagedorn et al., 2003; Foereid et al., 2004). However, for fertilization field-experiments these findings were questioned (Han et al., 2006; Khan et al., 2007). Results of lab-experiments can sometimes not be verified in the field (Oburger & Jones, 2009) due to indirect controls on decomposition and SOM stabilization (Sollins et al., 1996).

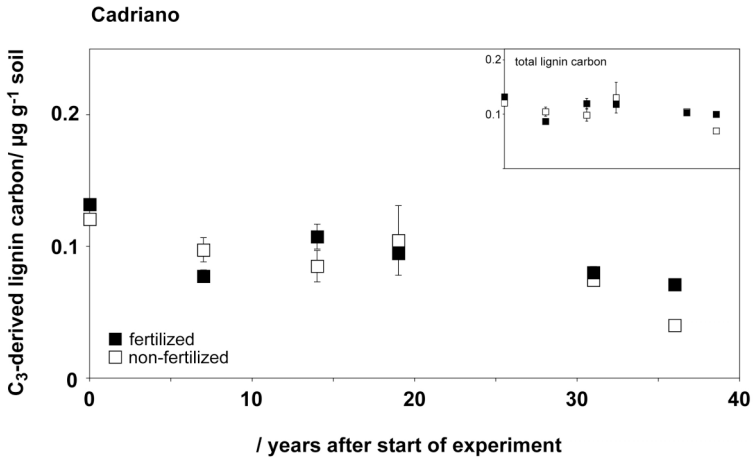


Figure 11 Comparison of the effect of mineral fertilization treatments on the decomposition of C_3-derived lignin carbon (C_{VSC}) in two soils of the Cadriano continuous maize experiment (manuscript II). In the 'fertilized' treatment 300 kg N ha^{-1} a^{-1} and 150 kg P ha^{-1} a^{-1} were applied to the maize crop. The non-fertilized treatment received no mineral fertilizer except nitrogen from atmospheric deposition which ranges between 10 to 25 kg N ha^{-1} a^{-1} in the region (Holland et al., 2005).

What might be the cause for no distinct mineral fertilization effect on decomposition of lignin? One explanation might be that, other than a blank treatment in a controlled laboratory study, the non-fertilized treatment was subjected to ambient atmospheric nitrogen deposition (Henriksen & Breland, 1999; Holland et al., 2005). It has been shown recently that it requires relatively low amounts of nitrogen to maintain the maximal rate of microbial decomposition (Schimel & Weintraub, 2003; Keeler et al., 2009). The ambient nitrogen deposition might have been high enough to level out any limiting effects on microbial populations in the field experiment.

4.3 Biomass incorporation did not enhance lignin decomposition

The return of harvest residues like cereal straw or maize stover (chopped leaves, stems, cobs) is a common management practice in arable soils of farming systems dominated by market crops such as small grain cereals or maize, in comparison to animal farm systems where harvest residues are used for animal forage. The incorporation of surplus crop residues was suggested as a potential measure for accumulating additional organic carbon in arable soil, with maximum incorporation rate of 10 t dry matter ha^{-1} a^{-1} (Smith et al., 2000).

In manuscript I it was demonstrated that additional inputs of maize stover after grain harvest resulted in an increase of 9.7 ± 2.2 t C ha^{-1} over 18 years, similar to the straw sequestration rates given by Freibauer et al. (2004).
In the study we focused on the possible priming effect that an additional biomass input might have on decay of organic matter, especially on lignin in these soils.

As shown in Figure 12, the C_3-derived lignin carbon concentrations did not significantly differ between treatments of low and high additional aboveground maize input over the period of the experiments in both soils, which indicates that in the long-term no priming effects can be measured in the amended soils. Treatment of additional aboveground maize biomass thus did not stimulate decay of old lignin as in positive priming (Kuzyakov et al., 2000).
Priming might have, however occurred in the short term, e.g. right after harvest residues were incorporated, and might then have been measured as higher microbial activities or increased rates of CO_2 formation. These effects however were not strong enough to impact the long-term decay of lignin significantly.

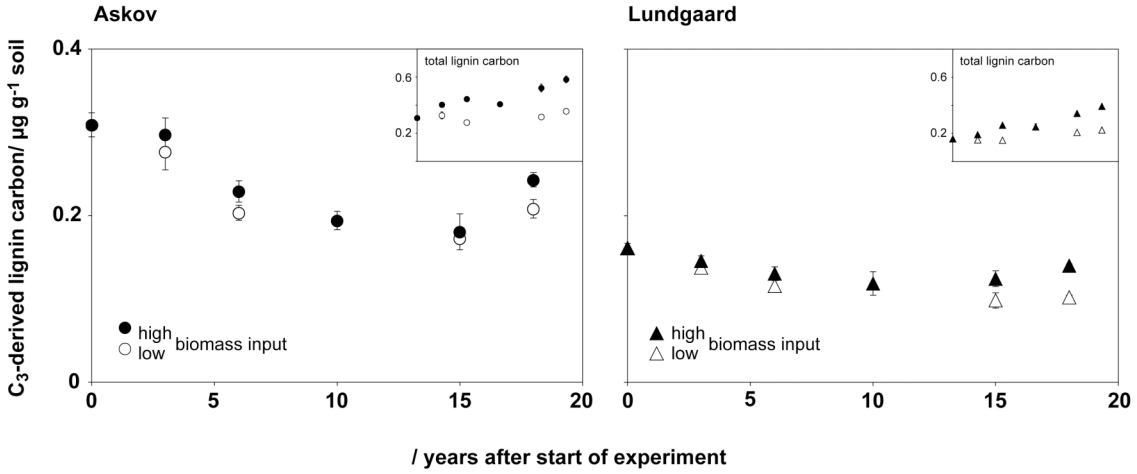


Figure 12 Comparison of the effect of biomass input treatments on the decomposition of C_3-derived lignin carbon (C_{VSC}) in two soils of the Askov continuous maize experiment (manuscript I). High biomass input denotes the treatment where above-ground maize biomass (maize stover) was incorporated into the soil (equivalent amount to 8 t ha^{-1} a^{-1}). Low biomass input denotes the treatment where only stubbles and below-ground maize biomass was incorporated (no samples available from the archives for year 10).

4.4 Lignin retention in silt-sized fraction and in particulate organic matter

Loss of lignin carbon within 18 years was smallest in the coarse free particulate organic matter (fPOM, Figure 13) and in the silt-sized fraction (free silt, Figure 13), suggesting that lignin might have been preserved or stabilized in these fractions. All other fractions showed relatively large losses of lignin carbon (Figure 13).

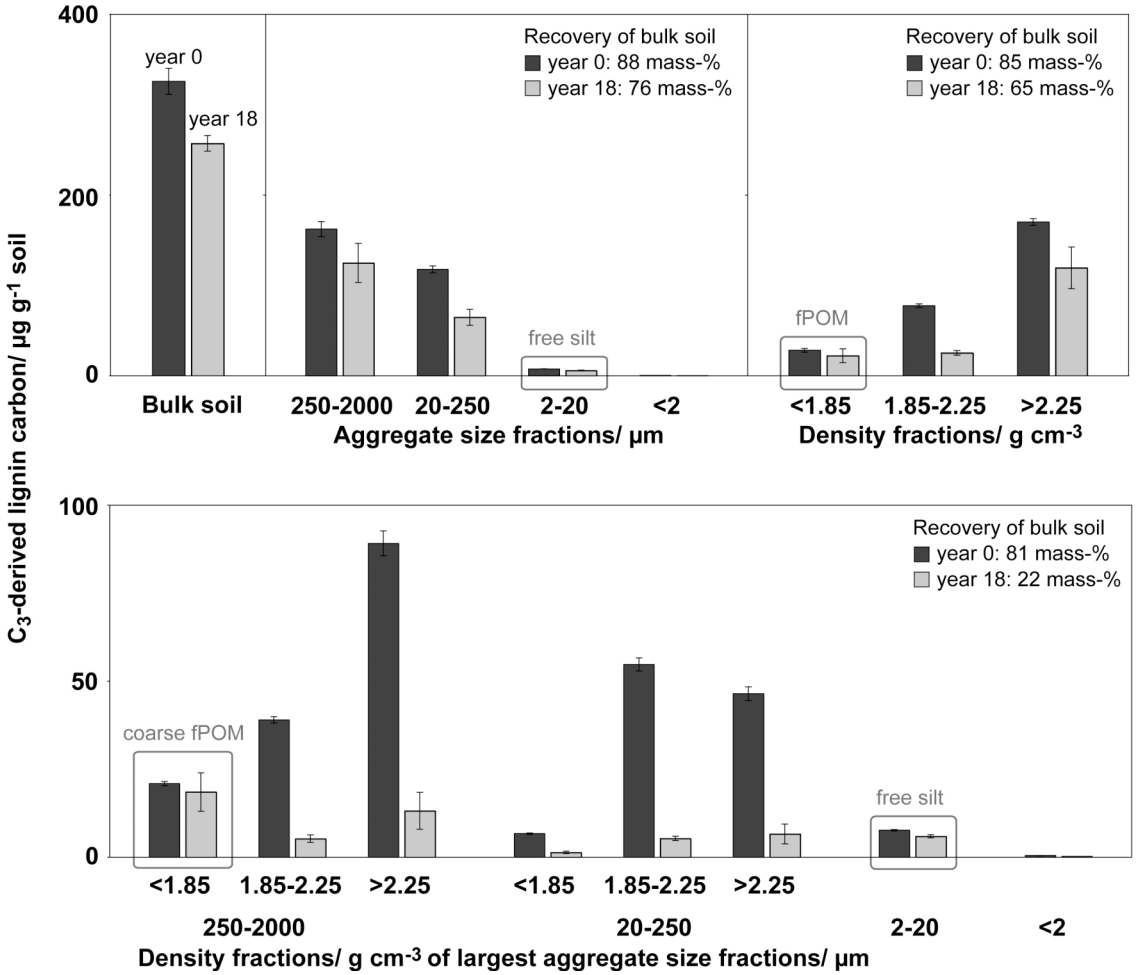


Figure 13 C_3-derived lignin carbon (C_{VSC}) in soil fractions of years 0 and 18 (manuscript III). Error bars represent $n = 3$ analytical replicates for each fraction. Mass recoveries of bulk soil lignin carbon concentration in the fractions were higher, when bulk soil was fractionated directly. Mass recoveries were low when the largest aggregate size fractions were subjected to further fractionation. Carbon losses as dissolved organic carbon (<0.45 μm) during the fractionation steps were tested as the cause, but were too low to account for the small mass recoveries.

Free particulate organic matter fractions (density class <1.85 g cm^{-3}) contained plant residues with recognizable plant cell walls, in early states of decomposition (see SEM images in Figure 14, in manuscript III and in the Appendix). Analysis of the mineral composition revealed that the fractions of a lighter density nevertheless contained approximately 65 mass-% mineral material (Table 2, manuscript III). The minerals appear as crusts on the organic matter (Figure 14, 1.), forming early stages of aggregates. The protection of the cell wall surfaces by minerals might be one of the reasons why lignin in free particulate organic matter decomposed relatively slowly within 18 years. Figure 14 presents two other reasons, (i) spatial inaccessibility within almost intact cell wall structures, where lignin in less accessible parts might be retained longer (Figure 14, 2.) or (ii) spatial inaccessibility at the molecular level, where more exterior polymer components might be oxidized first (Figure 14, 3.). The latter suggestion directly refers to the stabilization mechanisms of

selective preservation due to primary recalcitrance (von Lützow et al., 2006). As shown in the recent review by Marschner et al. (2008) recalcitrance is not relevant in the long-term. Our findings are in accordance with this conclusion: even if lignin might decompose relatively slowly in some soils, eventually it will decompose. The set of beliefs that lignin might be an especially recalcitrant component of soil organic carbon can be rather confidently abandoned with recent findings of lignin decaying faster than bulk SOC manuscripts I + II). The suggestions of figure 14 thus should not be seen as insisting on a long-term (centuries) retention of lignin in soils but rather as a way to help explain the - nevertheless – relatively slow decay within decades.

As pointed out by Heim and Schmidt (2007b) and Marschner et al. (2008), selective preservation through primary recalcitrance might not be the only way to protect lignin from degradation in soils. Protection in some form of aggregates as proposed in Figure 14 (1.) is thus a likely additional opportunity. It might be an aspect in a future study to trace lignin particularly in occluded particulate organic matter in aggregates.

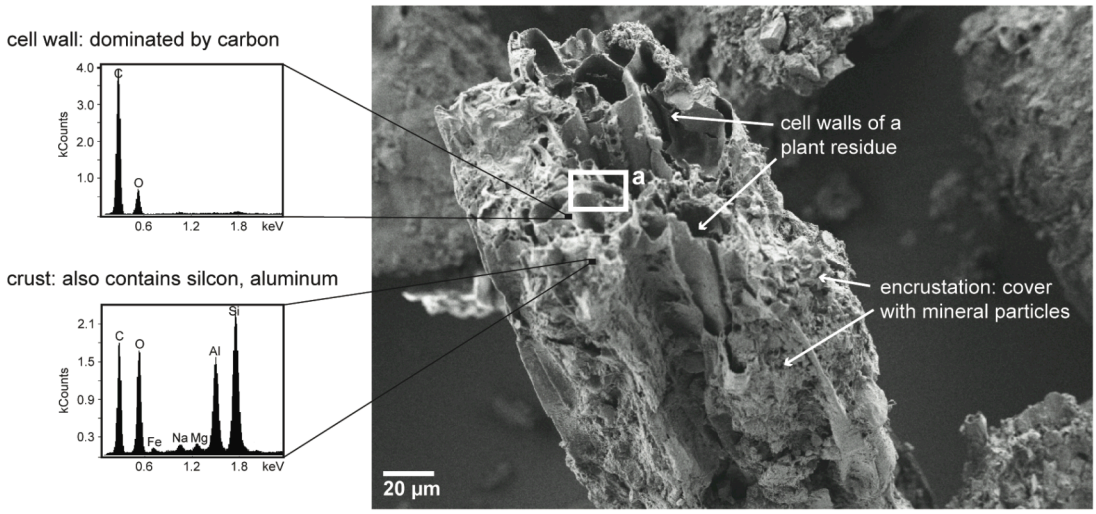
Consistent with the study of Heim and Schmidt (2007b), who demonstrated that C_3-derived lignin was preserved in the silt fraction of soil from the Rotthalmünster experiment, the results of manuscript III support the importance of the silt-size fraction for lignin retention (Figure 13). The silt fraction in manuscript III was free silt as opposed to silt bound in micro- or macro-aggregates. SEM images (Appendix) revealed that this silt was also organized as aggregates (silt-sized aggregates). Possible ways of lignin preservation in the silt fraction might thus be the protection by aggregation in the silt-size fraction or, as Heim and Schmidt (2007b) suggested, the interaction between lignin molecules and the mineral phase. Because silt-sized aggregates are the smallest aggregates, they might be least affected by aggregate turnover. This could subsequently result in the retention of organic matter occluded within these silt-sized aggregates. Thus, the best physical protection of organic matter might be in the smallest aggregates.

At this point in the interpretation of the results the limitations of soil fractions become clear. With the fractions alone it is not possible to directly address the mechanisms of stabilization, because one fraction, as presented e.g. by the scheme in Figure 9, cannot be representative of a mechanism. The fractions can provide information about the importance of certain soil components for stocks of lignin within the total soil or about lignin concentration changes, but the fractions cannot provide any mechanistic explanation of why the lignin is retained or lost in this fraction (lignin might even be redistributed between the fractions as discussed in manuscript III). The mechanisms thus have to be better explained with correlations, e.g. to factors such as oxide concentrations (Miltner & Zech, 1998) or with tracer studies that focus on aggregation.

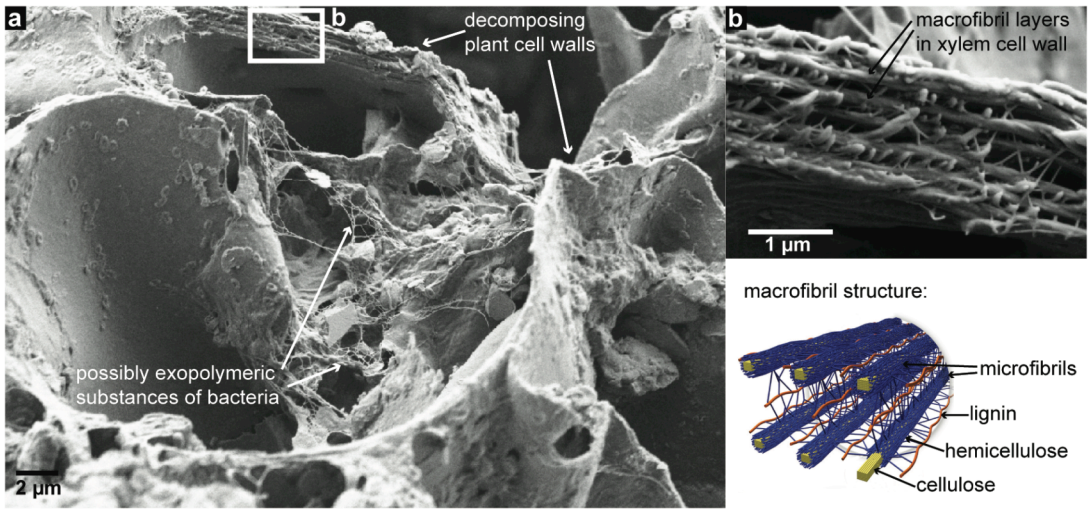
Figure 14 (next page) Lignin decay in soils may be slowed because of spatial inaccessibility of the macromolecule to decomposing enzymes at different scales (free particulate organic matter/aggregate, cell wall, molecule). Three possibilities of spatial inaccessibility for the lignin macromolecule are proposed and visualized. At the first level, free particulate organic matter might be protected from decay by a form of aggregation or partial occlusion within minerals, at the second level (cell wall) by physical protection through inaccessibility of certain parts of the cell walls and at the third level by chemical recalcitrance of the macromolecule.
The graphic of the macrofibril structure was created by the Genome Management Information System (2008), Oak Ridge National Laboratory, US Department of Energy Genome Programs, http://genomics.energy.gov. Lignin models were created by J. Ralph, and published in supplemental online material of Boerjan et al. (2003).

Hierarchy of spatial inaccessibility might control lignin decay

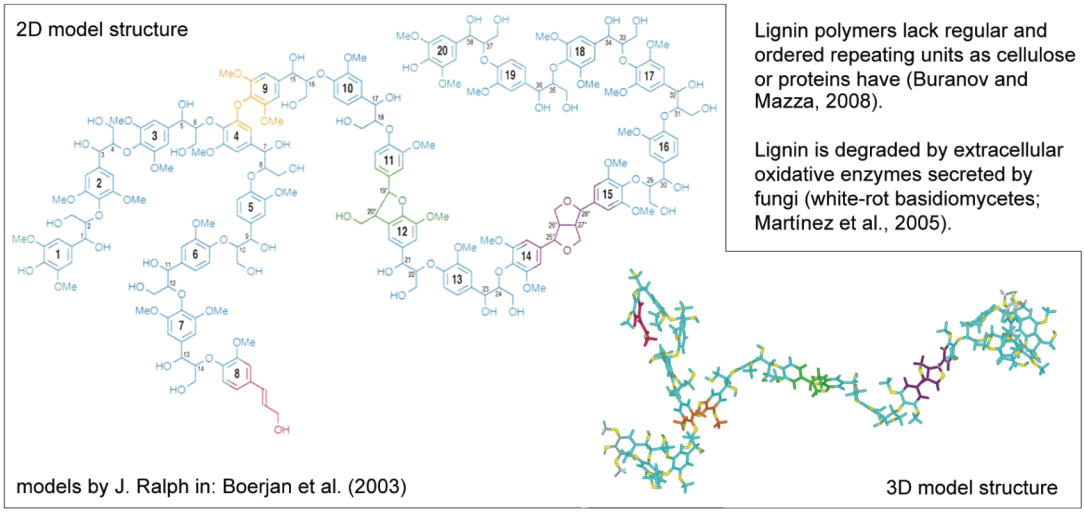
1. Plant residues in soil might be physically protected by a mineral crust or by occlusion in aggregates.



2. As a component of plant cell walls lignin might be less accessible in thick xylem cell walls.



3. Lignin is a complex three-dimensional polymer, enzymatic attack might start at outer parts.



5. Conclusions

1. Lignin dynamics were best described by double-exponential decay.

Lignin, like all other components of soil organic carbon, eventually decomposes. No specific recalcitrance exists for lignin. Timescales of decomposition are likely to be decades. Turnover times rely on one-pool models, whereas for lignin two pool-models might be more suitable: a fast pool of easily accessible lignin and a slow pool of less accessible lignin. With the soil samples from archives of two long-term field experiments we can cover a time scale of 18 and 36 years. Eventually, it would be necessary to continue measurements in these long-term experiments to verify the time scales of decay experimentally.

2. Nitrogen effects retarding lignin decomposition might not be transferred from the lab to the field scale.

In a natural agro-ecosystem, decomposition of lignin was less sensitive to mineral fertilization than suggested from previous findings in laboratory experiments. Over a period of 36 years, fertilization neither retarded nor enhanced the decomposition of lignin in the studied field experiment in comparison to a non-fertilized treatment. Although laboratory experiments are essential for studying mechanistic relationships, they should be complemented by field experiments. In order to provide evidence, lignin concentrations might be measured in field experiments where distinct nitrogen effects were found.

3. Priming of lignin decomposition by input of fresh biomass seemed not relevant in the long-term.

High levels of biomass input did not affect the decomposition dynamics of lignin, as no increase in decomposition could be observed over a period of 18 years for the studied experiment. Intensive cropping with biomass return does thus not lead to increased mineralization of older organic matter that might have been protected from decomposition e.g. by occlusion in aggregates.

4. Lignin seemed to be stabilized in the coarse light fraction and in the free silt fraction.

The study provides evidence that lignin might be protected from decomposition for two decades within mineral encrusted particulate organic matter or in silt-sized aggregates, supporting the proposed mineral interaction. Further labeling experiments with particulate organic matter (free or occluded in aggregates) from long-term experiments could help to elucidate how lignin can be retained in soils over decades.

6. Research perspectives

Applications

- Labeled lignin might be measured in other long-term field experiments (e.g. fertilization experiments, where nitrogen effect on soil organic carbon had been shown previously) and/or soils of different land uses (e.g. grassland or forest sols, which comprise more total soil organic carbon and more free particulate organic matter).

- A mass balance of decomposition might be generated that allows tracing quantitatively important organic compound classes from plant biomass input to soil organic carbon.

Stabilization mechanisms

- Aggregation and mineral encrustation, that were suggested to be involved in relatively slow lignin decay, could be explored by double labeling, where labeled occluded particulate organic matter in aggregates from one soil is introduced to a differently labeled soil in incubation or semi-field experiments. This approach would allow to directly study the stability of this occluded particulate organic matter and the lignin it contains.

- Measurement of enzymatic activity (Keeler et al., 2009) and of other microbiological parameters in soil fractions might help to better characterize lignin decay conditions.

Modeling

- The accumulated data of lignin decay in arable soils (Lobe et al., 2002; Dignac et al., 2005; Bahri et al., 2006; Rasse et al., 2006; Heim & Schmidt, 2007a; Heim & Schmidt, 2007b; Bahri et al., 2008; Hofmann et al., 2009a; Hofmann et al., 2009b) might be combined in a revised model to better define the proposed two pools (Rasse et al., 2006) on longer time scales.

Methods

- Enzymatic extraction of lignin from soil (Dignac et al., 2009) might be a methodological advancement for the quantification of lignin.

- Establishing a method for lignin monomer quantification and isotope analysis with HPLC-IRMS would help to avoid derivatization, which introduces additional carbon atoms that now require correction (Dignac et al., 2005).

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Part B Publications

Manuscript I

Lignin dynamics in two ^{13}C-labelled arable soils during 18 years

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Lignin dynamics in two ^{13}C-labelled arable soils during 18 years

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Summary

Lignin has long been considered a relatively stable component of soil organic matter. However, recent studies suggest that lignin may turn over within years to decades in arable soil. Here we analysed lignin concentrations in an 18-year field experiment under continuous silage maize, where two soils were sampled at six points in time. Our objectives were to examine the long-term dynamics of (i) lignin derived from a previous C3-vegetation and (ii) lignin derived from maize, as influenced by two levels of maize biomass input. Total lignin concentrations in soil were quantified by gas chromatography of lignin cupric oxide oxidation products. Compound-specific ^{13}C isotope analysis allowed discrimination between C3-derived lignin and maize-derived lignin. Degradation dynamics of C3-derived lignin were independent of biomass input level, suggesting that priming did not affect soil lignin concentrations. After 18 years, approximately two-thirds of the initial C3-derived lignin remained in the soils, whereas, on average, 10% of the recent maize-derived lignin input was retained. We suggest that lignin is effectively stabilized in these arable soils, although the mechanisms involved remain unclear.

Introduction

Lignin has long been regarded as one of the most stable components of soil organic matter (SOM) (Derenne & Largeau, 2001; Hatakka, 2001; Kögel-Knabner, 2002; Sanderman & Amundson, 2005). However, Kiem & Kögel-Knabner (2003) found that lignin did not accumulate in the refractory soil organic carbon (SOC) pool of soils of eight long-term arable experiments, but contributed mainly to the labile pool. Recent direct analytical evidence for fast lignin turnover has come from compound-specific studies of field experiments involving ^{13}C-labelled lignin (Dignac *et al.*, 2005; Heim & Schmidt, 2007a) determined 9 and 23 years after addition, respectively. Calculated lignin turnover times ranged from 10 to 40 years, assuming single exponential decay (Heim & Schmidt, 2007a). Rasse *et al.* (2006) modelled lignin turnover using data from a 9-year field experiment with annual soil sampling (Dignac *et al.*, 2005; Bahri *et al.*, 2006). Rasse *et al.* (2006) were able to describe their field data by a two-reservoir model in which lignin in plant residues turned over within less than 1 year and only 8% of the lignin from plant residues reached the SOC pool, where it was protected from further decomposition (turnover time of 19 years). To validate the suggested time scales of lignin turnover, ^{13}C-labelling experiments running over several decades and including frequent soil sampling are needed.

Lignin enters the soil via plant residues, in arable soils mainly by incorporation of crop residues after harvest. The annual quantity of these inputs can affect the organic carbon (OC) stock of the soil. If the quantity of the inputs is greater than the loss rate by decomposition, such inputs directly increase the SOC stock. However, there is also an indirect effect of plant residue input via the stimulation of microbial activity. Ocio *et al.* (1991) showed that the incorporation of straw caused a short-term increase in soil microbial biomass. This additional microbial activity might in turn enhance the decay of older SOC as in a positive priming effect (Kuzyakov *et al.*, 2000). Berg & McClaugherty (2003) also suggested that alternative C sources other than lignin might promote the degradation of lignin by several white rot fungi. We therefore hypothesize that the degradation of old lignin might be stimulated by the annual incorporation of crop residues (e.g. maize stover).

A methodology to study the effect of these crop residue inputs is the addition of a known amount of above-ground biomass (e.g. Bolinder *et al.*, 1999), i.e. similar to a spike in chemical

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analysis, on top of the unknown below-ground input of stubbles, roots and rhizodeposits. Resulting differences between the addition and no addition treatments can then be related directly to the amount of above-ground biomass entering the soil every year.

The spiking methodology can be coupled to natural ^{13}C abundance labelling of SOC. The label is achieved through a conversion from C3- to C4-vegetation, which introduces naturally ^{13}C-enriched plant residues to the soil (Balesdent *et al.*, 1987). After conversion to continuous C4-vegetation (e.g. maize in arable systems), no new C3-biomass is added to the soils and the C3-derived SOC will decrease or remain constant during the experimental period. Remaining C3-derived SOC represents organic moieties relatively resistant to degradation (i.e. stabilized in soil). Changes in the concentration of new, C4-derived SOC represent the retention of new biomass input by the soil. The dynamics of C3- and C4-derived SOC over time thus provide insight into the OC stabilization potential of the studied soils. Compound-specific isotope analysis of ^{13}C-labelled SOC allows tracking the fate of individual organic components such as lignin over decades.

In order to study the effects of crop residue incorporation on lignin concentrations in arable soils we examined (i) degradation of old C3-lignin and (ii) retention of new C4-lignin as influenced by two levels of above-ground biomass input. We used repeated sampling of two soils from an 18-year-old field experiment under continuous silage maize cropping.

Materials and methods

Soils

Soil was sampled from the soil archive of the continuous maize experiment at Askov, Denmark (55°28′N, 09°07′E; mean annual temperature 7.7°C; mean annual precipitation 862 mm; Christensen, 1997), which is a C3- to C4-vegetation conversion experiment. Silage maize is grown every year in open-ended cylinders (diameter 0.7 m, depth 0.5 m) inserted into the ground. In 1988 the cylinders were filled with four different soils sampled from the 0–25 cm horizon of arable fields from Danish experimental stations (Kristiansen *et al.*, 2005). Two soils, Lundgaard and Askov, had been cropped exclusively with C3-plants (mainly small grain cereals with barley as the dominant crop) before continuous silage maize (C4-plant) cropping was started in 1988. Before the conversion, the two soils had received C3-carbon input only from stubbles (length *c.* 10 cm) and below-ground plant biomass, the straw had been baled and removed from the fields. Only mineral fertilizers had been used. Both soils had been under arable cultivation for more than 100 years and the cereal cropping had been applied for many years before the soils were retrieved for the continuous maize experiment.

Lundgaard and Askov soils differ in texture and initial SOC content (Table 1). Thus the effect of soil texture cannot be studied independently from initial SOC or initial lignin content, the

Table 1 Chemical and physical characteristics of the experimental soils at the start of the experiment (1988, year 0)

			Soil texture				SOC			Lignin carbon (C_{vsc})	
		Bulk density[b]	Sand	Silt	Clay		Concentration[c]	$\delta^{13}C$[d]	C/N[c]	Concentration[c]	$\delta^{13}C$[c,e]
Soil description[a]	Origin[b]	/g cm^{-3}	/%			pH (H_2O)[b]	/mg g^{-1} soil	/‰ V-PDB	/mg C mg^{-1} N	/mg g^{-1} soil	/‰ V-PDB
Sand (Alfisol, Hapludalf)	Lundgaard	1.33	86.8	7.7	5.5	7.6	11.0 ± 0.3	−26.3 ± 0.4	10.2 ± 0.0	0.16 ± 0.01	−32.4 ± 0.5
Loamy sand (inceptisol, Ochrept)	Askov	1.12	64.6	21.2	14.1	6.4	25.6 ± 0.5	−27.8 ± 0.0	12.9 ± 0.4	0.31 ± 0.01	−32.6 ± 0.7

[a]Christensen (1997).
[b]Kristiansen *et al.* (2005).
[c]Mean ± standard error ($n = 6$ analytical replicates).
[d]Mean ± standard error ($n = 2$ analytical replicates).
[e]Corrected $\delta^{13}C$ of BSTFA according to Dignac *et al.* (2005).

finer textured soil (Askov soil) containing higher concentrations of SOC and lignin C (Table 1).

Records of bulk density were available for the start of the experiment only (Table 1). Changes in bulk density over the experimental period could thus not be taken into account. Results of this study are therefore presented in concentrations and not in overall stocks.

Treatments

At harvest, maize stems were cut and removed to leave stubbles of *c*. 4 cm length. Subsequently, two treatments were applied: (i) a small input treatment where the input of maize OC was derived solely from stubbles and below-ground biomass (roots and rhizodeposits) and (ii) a large input treatment ('spike') with an additional OC input of coarsely chopped above-ground maize biomass (stems, leaves, cobs) incorporated into the soils in the autumn (800 g dry matter m^{-2} $year^{-1}$; Kristiansen *et al*., 2005). Table 2 lists characteristics of the C3- and C4-input biomass from plants that were grown in the experimental soils. Only samples for aboveground biomass were available, so the concentrations of OC or lignin in roots and stubbles could not be determined.

Soil sampling

Representative bulk soil samples were taken every 2–3 years from the experimental soil horizon (0–20 cm) in the spring prior to planting, air-dried, sieved to < 2 mm and archived. For our study, we sub-sampled archived soil from 1988 (year 0, control), 1991 (year 3), 1994 (year 6), 1998 (year 10; only available for 'large input'), 2003 (year 15) and 2006 (year 18).

Soil organic carbon analysis

Carbon concentrations were determined at least in triplicate (three sub-samples of one homogenized field sample per treatment and date; six analytical replicates exist for years 0 and 3) on a CHNS elemental analyser (Vario EL, Elementar Analysensysteme, Hanau, Germany) after removal of carbonates by fumigation with concentrated hydrochloric acid (6 h, Harris *et al*., 2001). Stable carbon isotopic composition was measured in duplicate (two sub-samples of one homogenized field sample per treatment and date) using an elemental analyser coupled on-line to an isotope ratio mass spectrometer (EA-IRMS; NA 2500, CE-Instruments, Rodano, Milan, Italy; Delta plus, Finnigan MAT, Bremen, Germany; Kompetenzzentrum Stabile Isotope, Göttingen, Germany). Analytical precision was 0.1‰ V-PDB.

Lignin extraction from soil

Lignin was extracted at least in triplicate from air-dried, finely ground soil (three sub-samples of one homogenized field sample per treatment and date; six analytical replicates exist for years

Table 2 Characteristics of the above-ground plant biomass input

	Organic carbon (OC)			Lignin carbon (C_{vsc})	
	Concentration[a]	$\delta^{13}C$[a]	C/N[a]	Concentration[a]	$\delta^{13}C$[a,b]
Plant biomass	/mg C g^{-1} plant dry matter	/‰ V-PDB	/mg C mg^{-1} N	/mg C_{vsc} g^{-1} plant dry matter	/mg C_{vsc} g^{-1} plant OC	/‰ V-PDB
Barley straw (C3)	430.6 ± 1.3	−25.4 ± 0.6	68.2 ± 1.9	26.1 ± 0.5	60.5 ± 1.2	−35.7 ± 0.5
Maize stover (C4)	421.9 ± 1.1	−12.2 ± 0.3	39.7 ± 1.3	24.0 ± 0.5	57.0 ± 1.2	−18.9 ± 0.1

[a]Mean ± standard error ($n = 3$ analytical replicates of one pooled barley straw sample (years 1989–1992) of the Askov experimental fields or of one maize stover sample (year 2001) of the continuous maize experiment).
[b]Weighted average of $\delta^{13}C$ of lignin monomers, corrected for $\delta^{13}C$ of BSTFA according to Dignac *et al*. (2005).

0 and 3) using alkaline cupric oxide oxidation (Hedges & Ertel, 1982) in a microwave digestion system (Goñi & Montgomery, 2000) as adapted by Heim & Schmidt (2007a). In the extracts, the oxidation products vanillyl, syringyl and cinnamyl phenols were quantified and their sum used as an indicator of lignin (VSC). We are aware that VSC lignin is not a quantitative measure of the actual lignin concentration. However, in the absence of better analytical alternatives the cupric oxide oxidation method is widely used for soils. Lignin C (C_{VSC}) was calculated from the molecular formulae of the individual lignin oxidation products (for monomers see Heim & Schmidt, 2007a), which contain between 55–65 mass-% C. Quantification of VSC was conducted by gas chromatography coupled to a flame ionization detector (GC-FID; HP 6890N Plus, Agilent Technologies, Wilmington, DE, USA) with a DB-5MS column, length 50 m, inner diameter 0.2 mm, film thickness 0.33 μm (Agilent J & W, Folsom, CA, USA) connected to a G1530N capillary flame ionisation detector (Agilent Technologies); temperature programme: 100°C, ramp to 160°C at 3°C minute^{-1}, hold for 5 minute, ramp to 250°C at 3°C minute^{-1}, ramp to 320°C at 10°C minute^{-1}, hold for 10 minutes. In order to volatilize lignin monomers from extracts (dissolved in ethyl acetate), BSTFA/TMCS 99:1 derivatization agent was added 1:1 (vol.). Quantification was achieved using calibration curves of external lignin monomer standards. To correct for losses during sample preparation, cinnamic acid and ethyl vanillin were used as internal standards (Heim & Schmidt, 2007a). The average relative standard error for lignin analysis at the GC-FID was 5%.

Compound-specific stable carbon isotope analysis

Compound-specific isotope analysis for lignin monomers (Goñi & Eglinton, 1996) was conducted in duplicate for each soil extract using gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS; gas chromatograph HP 6890N Plus, Agilent Technologies, interface Combustion III, ThermoQuest-Finnigan, Breman, Germany and isotope ratio mass spectrometer MAT 252, Finnigan, Bremen, Germany). Column, temperature programme and derivatization were as described above for lignin monomer quantification by GC-FID. For GC-C-IRMS measurements we used the alkanes n-20 and n-24 as internal standards (Heim & Schmidt, 2007a). Correction for the shift in the isotopic composition by adding trimethylsilyl C during derivatization (BSTFA/TMCS 99:1) was conducted according to the mass balance equation by Dignac *et al*. (2005) (Equation (1)):

$$\delta_{UD} = \frac{n_D}{n_{UD}}\delta_D - \frac{n_{BSTFA}}{n_{UD}}\delta_{BSTFA}, \qquad (1)$$

where δ_{UD} and δ_D are the stable C isotopic ratios of the underivatized and derivatized phenol (as measured by GC-C-IRMS), and n_{UD} and n_D are the number of C atoms in the underivatized and derivatized phenol. n_{BSTFA} is the number of trimethylsilyl C atoms added to the phenol from BSTFA. Analytical precision for $\delta^{13}C$ analysis of lignin at the GC-C-IRMS was 0.6‰ V-PDB.

The $\delta^{13}C$ of added trimethylsilyl C was analysed on-line with the GC-C-IRMS by measuring the $\delta^{13}C$ first of methanol and second of methanol derivatized with BSTFA from the headspace and then solving the mass balance equation for BSTFA (Equation (2)):

$$\delta_{BSTFA} = \frac{(n_D \times \delta_D) - (n_{UD} \times \delta_{UD})}{n_{BSTFA}}, \qquad (2)$$

where n_D and n_{UD} are the number of C atoms in the derivatized ($n_D = 4$) and underivatized methanol ($n_{UD} = 1$), δ_D and δ_{UD} are the stable C isotopic ratios of the derivatized and underivatized methanol ($\delta_D = -37.6$ and $\delta_{UD} = -30.3$ as measured by GC-C-IRMS), and n_{BSTFA} is the number of C atoms in the trimethylsilyl group of BSTFA ($n_{BSTFA} = 3$).

Calculation of C3- and C4-derived lignin

The fraction of new, C4-derived lignin C (C4-C_{VSC}) within total lignin C (C_{VSC}) was calculated by adapting the formula for SOC established by Balesdent & Mariotti (1996), as proposed by Dignac *et al*. (2005) and Heim & Schmidt (2007a) (Equation (3)):

$$F_{newCVSC} = \frac{\Delta\delta^{13}C_{soils}}{\Delta\delta^{13}C_{plants}}, \qquad (3)$$

where $F_{newCVSC}$ is the fraction of new, C4-derived lignin C, $\Delta\ \delta^{13}C_{soils}$ is the difference between the delta values (‰ V-PDB; determined by GC-C-IRMS) of lignin extracted from soil before and after the conversion and $\Delta\ \delta^{13}C_{plants}$ is the difference between the delta values of lignin extracted from the input vegetation (Table 2). Quantities of C4-derived lignin were calculated by multiplication of $F_{newCVSC}$ with the lignin concentration determined by GC-FID. C3-derived lignin is the difference between total lignin and C4-derived lignin.

Statistics

Concentrations and $\delta^{13}C$ values are given as the mean with the standard error. The standard errors of SOC and C_{VSC} concentrations and the $\delta^{13}C$ values of C_{VSC} represent the analytical error of six analytical replicates (years 0 and 3) or three analytical replicates (years 6, 10, 15, 18) of one homogenized archived soil sample for each treatment and sampling date. The standard errors of the $\delta^{13}C$ values of SOC represent the analytical error of two analytical replicates. Error propagation calculations were always included. The significance of differences was tested by unpaired *t*-tests (two-sample equal variance or two-sample unequal variance if applicable).

Results and discussion

Soil organic carbon

Over the course of the experiment, SOC concentrations increased in both soils when above-ground maize biomass inputs were large and remained almost constant when inputs were smaller (Figure 1e). Contrary to our finding, Kristiansen *et al.* (2005) had observed that SOC stocks of both soils (especially Lundgaard) and both treatments increased during the first 14 years of the continuous maize experiment. While the trend of SOC increase with larger input can be confirmed with the present study, the trend for increased SOC concentrations with smaller input could not be confirmed for the 18-year period.

Contribution of lignin carbon to soil organic carbon

The proportion of lignin C (C_{VSC}) in SOC ranged between 11.3 ± 0.8 and 27.3 ± 0.6 mg C_{VSC} g^{-1} SOC (Figure 1a) and was comparable to values found in other studies with arable soils where lignin extraction was also conducted using the cupric oxide oxidation method (Guggenberger *et al.*, 1994; Lobe *et al.*, 2002; Kiem & Kögel-Knabner, 2003; Dignac *et al.*, 2005; Bahri *et al.*, 2006; Heim & Schmidt, 2007a).

The proportion of C_{VSC} in SOC was increased with large input in comparison with smaller input and was overall greater for the coarse textured Lundgaard soil (Figure 1a). However, the Lundgaard soil contained less SOC (Figure 1e) and thus also less C_{VSC} per gram soil than the finer textured Askov soil. The effect of texture could not be evaluated independently from SOC concentrations because both soils varied in both factors (Table 1).

Most importantly, during the course of 18 years, the SOC of both soils generally became enriched in C_{VSC} (Figure 1a). Large crop residue input approximately doubled C_{VSC} proportions. Small crop residue input resulted in a small increase that was significant only for the Lundgaard soil (Figure 1a). The enrichment resulted from the increase in absolute C_{VSC} soil concentrations while changes in SOC concentrations were relatively small (Figure 1e).

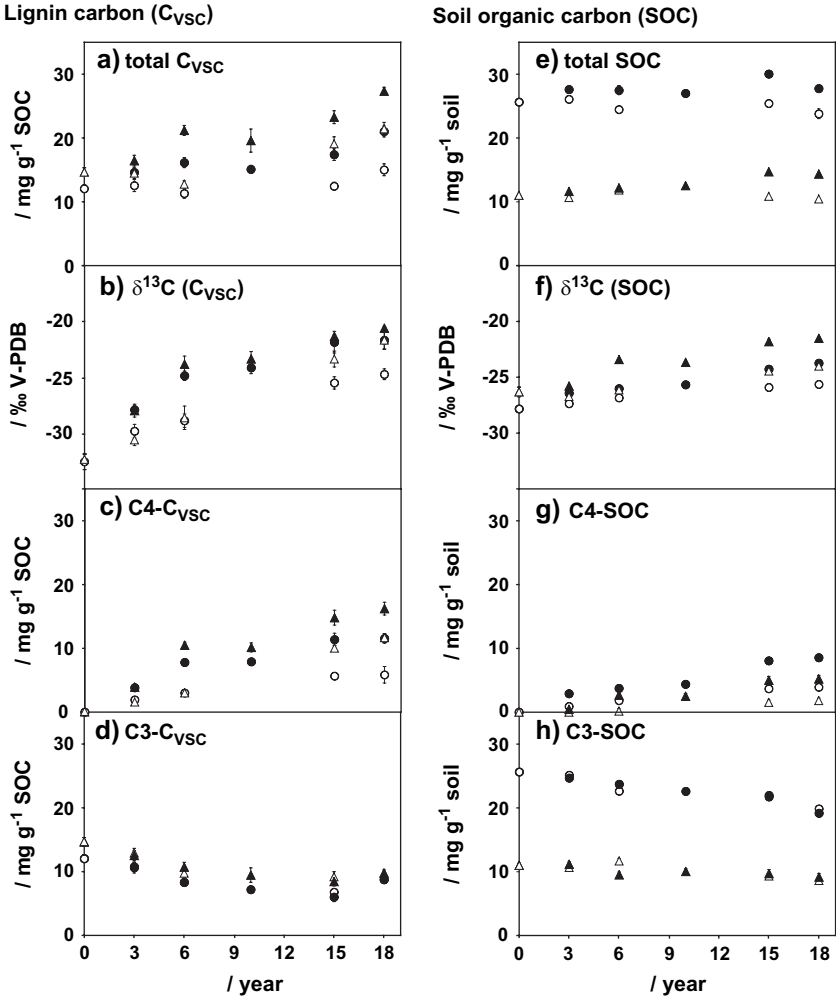

Figure 1 Lignin and soil organic C dynamics in the Askov continuous maize experiment over 18 years. Lundgaard soil: ▲ large input, △ small input. Askov soil: ● large input, ○ small input. Large input (▲●) denotes the treatment where above-ground maize biomass (0.8 kg dry matter m^{-2} a^{-1}) was incorporated into the soil. Small input (△○) denotes the treatment where only stubbles and below-ground maize biomass were incorporated (no samples available for year 10). The standard errors represent the analytical error of six analytical replicates (years 0 and 3) or three analytical replicates (years 6, 10, 15, 18) of one homogenized archived soil sample for each treatment and sampling date. (a) total lignin C (C_{VSC}), (b) $\delta^{13}C$ values of C_{VSC}, corrected according to Dignac *et al.* (2005), (c) new, maize-derived lignin C (C4-C_{VSC}), (d) old, C3-derived lignin C (C3-C_{VSC}), (e) total soil organic C (SOC), (f) $\delta^{13}C$ values of SOC, (g) new, maize-derived soil organic C (C4-SOC), (h) old, C3-derived soil organic C (C3-SOC).

The enrichment in C_{VSC} contrasts with the results of Bahri *et al.* (2006), who found almost constant C_{VSC} proportions over the course of a 9-year experiment of continuous grain maize. The enrichment in C_{VSC} could result from a greater proportion of C_{VSC} in maize plant inputs. However, for above-ground inputs we could not find greater proportions of C_{VSC} in biomass OC (Table 2). Unfortunately, data for C_{VSC} in below-ground inputs could not be obtained. Therefore we could not determine if the enrichment could be attributed to a greater proportion of C_{VSC} in below-ground inputs. A second possible explanation for the observed larger proportions of C_{VSC} in our 18-year experiment could be the preferential retention of C_{VSC} in these soils. However, a preferential retention of lignin compared with total OC would be in contrast to previous findings of Kiem & Kögel-Knabner (2003), Dignac *et al.* (2005) and Heim & Schmidt (2007a), who provided evidence that lignin turns over faster than SOC.

Mass balance of above-ground maize biomass input: determining the retention of new C4-lignin carbon (C4-C_{VSC})

The data from the Askov continuous maize field experiment allowed us to conduct a simple mass balance to determine the actual retention of recent C4-C_{VSC} and C4-OC from above-ground biomass inputs. Spiking the soil with 800 g dry matter m^{-2} $year^{-1}$ of above-ground maize biomass each year led to an annual input of 19 g C4-C_{VSC} m^{-2} and 338 g C4-OC m^{-2}, which accumulated 0.35 kg C4-C_{VSC} m^{-2} and 6.1 kg C4-OC m^{-2} within 18 years. Of these cumulated inputs, the soil retained, on average, 35 ± 9 g C4-C_{VSC} m^{-2} ($n = 2$, Lundgaard and Askov soil) and 970 ± 215 g C4-OC m^{-2} (Figure 2, calculations based on data presented in Figure 1b,c,f,g). This corresponds to an average retained fraction of $10 \pm 3\%$ for C4-C_{VSC} and $16 \pm 4\%$ for C4-OC during 18 years.

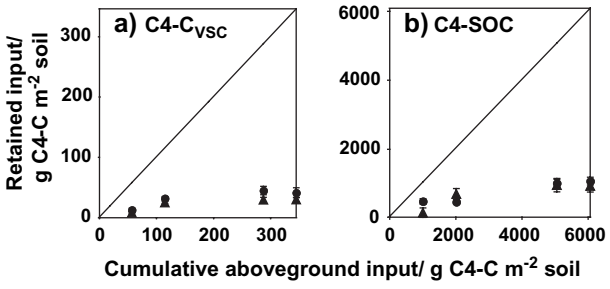

Figure 2 Retention of new C4-derived C (C4-C) from above-ground maize biomass input in the soils (top 20 cm) in relation to the cumulative C input from above-ground maize biomass during the experimental period of 18 years. Retained C4-C stocks from above-ground maize biomass were calculated as the difference between the C4-C stocks of large and small input treatments ('spiking'). ▲ Lundgaard soil, ● Askov soil. Error bars represent the standard error ($n = 3$ analytical replicates).

The average value of *c.* 10% C4-C_{VSC} retention was in close agreement with the 8% lignin retention from undecomposed plant residues proposed in the model by Rasse *et al.* (2006). Similarly, the average retention of maize-derived SOC of 16% was in agreement with, but slightly larger than, that found by Bolinder *et al.* (1999), who reported 12.2% as an average fraction of shoot-derived C retained as SOM. In comparison, the retention of C4-C_{VSC} from above-ground biomass tended to be smaller than C4-OC retention, pointing to a preferential degradation of lignin compared with OC, which supports the results of Dignac *et al.* (2005) and Heim & Schmidt (2007a). Both studies provided evidence that old C3-lignin was preferentially degraded compared with old C3-SOC. With the Askov continuous maize experiment we can now show a similar effect for recent C4-lignin inputs. This shows that relative stability of lignin in comparison with SOC was similar for recent biomass inputs and for old SOM. This result suggests that recent inputs of C4-lignin or C4-OC and older C3-lignin or C3-SOC were probably subjected to similar stabilization mechanisms.

Increasing soil carbon stocks by crop residue incorporation?

Freibauer *et al.* (2004) list a large number of possible C sequestration measurements in soil, one of which is 'crop residues', which in this case means incorporation of surplus cereal straw. Freibauer *et al.* (2004) give two estimates for potential C sequestration rates from (cereal straw) crop residues in arable soil (0.7 and 0.2 ± 0.1 t C ha^{-1} $year^{-1}$). The soils of the Askov continuous maize experiment sequestered a maximum of 9.7 ± 2.2 t ha^{-1} in 18 years (Figure 2), which is comparable with the annual rates given by Freibauer *et al.* (2004). However, Figure 2 shows that saturation effects occur and that initial sequestration rates cannot be projected linearly into the future.

Dynamics of C3-lignin decomposition

Figure 1d presents the proportion of C3-derived lignin C (C3-C_{VSC}) in total SOC, which generally decreased over the course of the experiment, faster in the first decade and slowing down in the second decade. Even so, after 18 years a large percentage of the initial C3-C_{VSC} was still present in the soils irrespective of soil type or biomass input (Figure 1d). On average for all soils studied, approximately two-thirds of the initial C3-C_{VSC} remained after 18 years. This is much larger than in the arable soil studied by Heim & Schmidt (2007b), which contained only 27% of the initial C3-C_{VSC} after 23 years. The large remaining percentage of C3-C_{VSC} after almost two decades may point to an effective stabilization of a part of old lignin moieties. However, this slow degradation of lignin has to be considered in the context of slow C3-SOC mineralization (Figure 1h). In comparison to the remaining C3-C_{VSC}, the remaining C3-SOC concentration in soils was slightly larger

after 18 years (Figure 1h), suggesting that old C3-C_{VSC} was replaced faster than old C3-SOC. This was a consistent trend throughout the experiment. To visualize these findings, we plotted percentages of remaining old C3-C_{VSC} against percentages of old C3-SOC for all samples analysed during the experimental period (Figure 3).

The low C3-SOC mineralization can probably be attributed to the stability of the SOC pool in these arable soils, which had been under intensive cereal cropping (with mineral fertilizer and removal of crop residues) for a long period before the experiment was initiated. Intensive cropping could have resulted in mineralization of most OC inputs, leaving solely SOC that was already stabilized.

The driving mechanisms for the observed stabilization of older organic matter might be related to, for example, chemical recalcitrance or organo-mineral interactions (Christensen, 1996; von Lützow *et al*., 2006; Marschner *et al*., 2008) and/or to the activity and community structure of the decomposer microorganisms (Hatakka, 2001; Sanderman & Amundson, 2005). Revealing the underlying mechanisms was beyond the focus of this paper, but should be addressed in the future.

Effect of biomass input on old C3-lignin carbon

The decrease of C3-C_{VSC} and C3-SOC appeared to be unaffected by the additional input of above-ground maize biomass in the long term (Figure 1d,h); no significant trends could be found. Generally, we can thus state that the expected positive priming of the old C3-C_{VSC} or C3-SOC resulting from the large biomass input could not be observed. If such a priming effect exists, it will be a short-term effect only, occurring directly after the incorporation of the biomass and the effect will thus not be detectable in the long term.

In the context of the experimental design, it can be added that the start of the experiment, i.e. after the conversion from small grain cereal to maize cropping, did not induce drastic changes in total SOC concentrations (Figure 1e). This supports the notion that soils were near steady state conditions for OC inputs and SOC levels, especially with the small biomass input treatment.

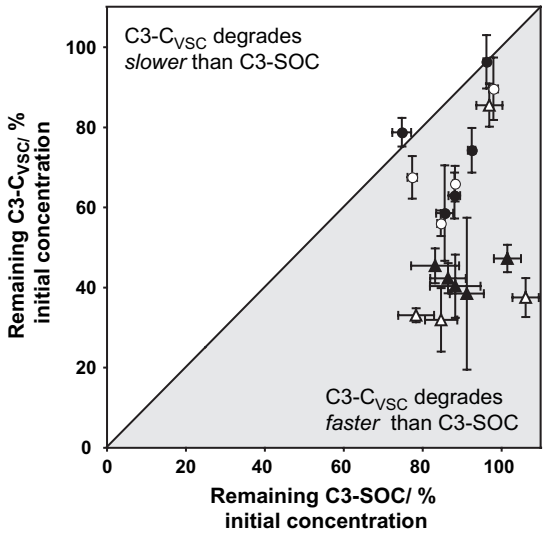

Figure 3 Percentages of remaining initial soil concentrations of C3-C_{VSC} plotted against those of C3-SOC in soil samples during the experimental period. Lignin was more susceptible to degradation than SOC. Lundgaard soil: ▲ large input, △ small input. Askov soil: ● large input, ○ small input. Error bars represent the standard error ($n = 3$ analytical replicates).

Conclusions

To the best of our knowledge, this is the first study that tracks ^{13}C-labelled lignin in an arable soil field experiment over 18 years. The data can be interpreted in terms of lignin degradation and stabilization and thus contribute to knowledge of the dynamics of lignin over long periods.

The three main conclusions are that (i) after 18 years of continuous maize cropping, approximately two-thirds of the initial old C3-derived lignin were still present in these soils, suggesting that lignin had been stabilized effectively, although underlying mechanisms remain unclear, (ii) levels of biomass input did not affect the degradation dynamics of old C3-derived lignin, priming from biomass inputs could not be observed over a period of 18 years, and (iii) the results give evidence for preferential degradation not only of old, C3-derived lignin carbon but also of recent, C4-derived lignin carbon over C3- and C4-SOC.

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Manuscript II

Mineral fertilization did not affect decay of old lignin and SOC in a ^{13}C-labelled arable soil over 36 years

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Mineral fertilization did not affect decay of old lignin and SOC in a ^{13}C-labeled arable soil over 36 years

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Abstract. Retardation of soil organic carbon (SOC) decay after nitrogen addition to litter or soil has been suggested in several recent studies and has been attributed to a retardation in lignin decay. With our study we tested the long-term effect of mineral fertilization (N+P) on the decay of the SOC component lignin in arable soil. To achieve this, we tracked ^{13}C-labeled lignin and SOC in an arable soil that is part of a 36-year field experiment (conversion from C_3 to C_4 crops) with two mineral fertilization levels. We could show that fertilization neither retarded nor enhanced the decay of old SOC or lignin over a period of 36 years, proposing that decay of lignin was less sensitive to fertilization than previously suggested. However, for new, C_4-derived lignin there were indications that decay might have been enhanced by the fertilization treatment, whereas decay of new SOC was unaffected.

1 Introduction

Mineral fertilization, specifically nitrogen fertilization, was already discussed in the 1950s as a potential means to raise soil organic matter concentrations in arable soils by increasing the amount of plant biomass returned to the soil (Allison, 1955). However, net storage of SOC is a balance between biomass input and decay (mineralization). Because soil microorganisms compete for nutrients with plants, the addition of mineral fertilizer might not only increase plant biomass production but also microbial biomass and microbial activity (Wang and Bakken, 1997). The latter effect could enhance the decay of soil organic matter. This hypothesis has recently been supported by the study of Khan et al. (2007) who showed that high mineral fertilization (NPK) led to significant losses of soil organic carbon during 51 years of continuous maize cropping at the Morrow plots (Illinois, USA). Even so, the evidence for changes in SOC due to fertilization with nitrogen is contradictory – especially for agricultural soils, whereas in forest soils nitrogen enrichment seems to suppress carbon loss (Reay et al., 2008). Fog (1988), Henriksen and Breland (1999) and Kuzyakov et al. (2000) give examples for a retardation of SOC decay under nitrogen fertilization, which was termed "negative priming effect" (Kuzyakov et al., 2000). In his review, Fog (1988) found that nitrogen has either a retarding or no effect on microbial activity and SOC decay in the long term. Retardation of decay after nitrogen addition was mainly reported for organic matter with high C/N ratios and high lignin contents. Fog (1988) suggested three explanations for the retardation of decay after nitrogen addition: (I) nitrogen might promote certain types of decomposer microorganisms on the expense of others, (II) nitrogen might block the production of certain enzymes of decomposer microorganisms, (III) amino compounds might condense with polyphenols to form toxic or inhibitory products. Especially the second explanation seems well supported by the literature. Already Keyser et al. (1978) found that the basidiomycetes *Phanerochaete chrysosporium* and *Trametes versicolor* produced lignin-degrading enzymes only when nitrogen levels were low. Similarly, Carreiro et al. (2000) found that the activity of lignin-degrading phenol oxidase declined in response to nitrogen. On the basis of these findings by Keyser et al. (1978), Fog et al. (1988) and Carreiro et al. (2000), recent studies suggested that a retardation of SOC mineralization under nitrogen additions might be due to the retardation of lignin decay (Hagedorn et al., 2003; Foereid et al., 2004). However, a direct effect of nitrogen, or mineral fertilization in general, on lignin decay has not yet been shown in long-term field experiments. Our objective was therefore to test if mineral fertilization might reduce lignin decay over a long time period in a field experiment.

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Recently, the study of lignin decay on a molecular level has improved through compound-specific stable isotope analysis of isotopically labeled lignin biomarkers (Dignac et al., 2005; Heim and Schmidt, 2007a). This method provides the opportunity to study the decay of the SOC component lignin in long-term field experiments (Bahri et al., 2006; Hofmann et al., 2009). To achieve this, we tracked ^{13}C-labeled lignin and SOC in an arable soil that is part of a 36-year arable soil field experiment with two mineral fertilization levels.

2 Materials and methods

2.1 Soils and treatments

For our study we made use of archived soil samples from a long-term field experiment of continuous silage maize cropping initiated in 1966 by Giovanni Toderi (Cadriano field experiment, University of Bologna, Italy; 44°32′51″ N, 11°23′56″ E, mean annual temperature 11°C, mean annual precipitation 650 mm). The crops grown on this site previous to the start of the experiment were plants with a C_3-photosynthetic pathway (e.g. wheat, alfalfa, beets), which have a lower natural abundance of the stable carbon isotope ^{13}C in comparison with plants that have a C_4-photosynthetic pathway such as maize. The continuous cropping of maize therefore introduced naturally ^{13}C enriched organic carbon to the soil (Balesdent et al., 1987), which can be used as a label to distinguish new, C_4-derived (= maize-derived) and old, C_3-derived SOC. The experimental soil was classified as Typic Udochrept (USDA, 1975). Gioacchini et al. (2007) provide chemical and physical characteristics of the soil: pH (H_2O) 6.9; soil organic carbon 8.5 g kg^{-1}; total nitrogen 1.1 g kg^{-1}; cation exchange capacity 16.5 cmol$_c$ kg^{-1}; sand 56%, silt 16%, clay 28%. The experiment includes two treatments, (I) conventional mineral fertilization (300 kg N ha^{-1}a^{-1}, 150 kg P ha^{-1}a^{-1}), which will be called "fertilized treatment" in the following and (II) no mineral fertilization (0 kg N ha^{-1}a^{-1}, 0 kg P ha^{-1}a^{-1}), which will be called "non-fertilized treatment". Atmospheric nitrogen (N) deposition ranges between 10 to 25 kg N ha^{-1}a^{-1} in the region (for the time period 1978–1994, Holland et al., 2005), thus the non-fertilized treatment does not represent a total absence of N inputs but rather an input by atmospheric deposition as it might be typical for large parts of Europe (Holland et al., 2005). The fertilization treatment was carried out with half the dose at sowing and the other half at the four-leaf stadium of maize plants.

2.2 Sampling of soil and plant biomass

The experiment was started in 1966 with two replicate plots per treatment. The plots were sampled in the years 1973, 1980, 1985, 1997, 2002 and the samples were archived. As no archived sample of the plots was available for year 1966, we used the sample of 1973 from a simultaneous continuous wheat experiment at the same experimental site to represent the initial conditions (C_3-plant input) in 1966. The soils were sampled after harvest (end of September for maize plots, mid-July for wheat plots) within the plow horizon with an electric auger to a depth of 25 cm until 1992, afterwards to a depth of 35–40 cm. For each plot four sub-samples were taken. The sub-samples were mixed, air-dried, ground and sieved to 2 mm. Plowing depth at the experimental field site was relatively deep (40–50 cm), as it is common in Mediterranean agriculture. Plant samples were collected at plant physiological maturity during harvest, when also total grain and stover yields were recorded. Unfortunately only aboveground plant biomass was sampled and archived, therefore no samples from belowground plant biomass were available for analysis.

2.3 Chemical analysis

Soil and plant samples were analyzed for carbon and nitrogen concentrations with an elemental analyzer (EA, Thermo Electron EA 1110, Germany). Stable carbon isotope composition (δ^{13}C) was determined using an elemental analyzer (EA, Thermo Electron EA 1110, Germany) coupled to a continuous flow-isotope ratio mass spectrometer (CF-IRMS, Delta Plus Thermo Electron, Germany). Lignin was extracted from soil and plant samples by cupric oxide oxidation (Hedges and Ertel, 1982; Goñi and Montgomery, 2000; as adapted by Heim and Schmidt, 2007a), which is a standard method for lignin analysis in soils and sediments. The oxidation products (lignin monomers vanillyl (V), syringyl (S) and cinnamyl (C) phenols) were quantified in the extracts by gas chromatography using a flame ionization detector (GC-FID, Agilent Technologies HP 6890N Plus, USA). The oxidation products are lignin-specific and their sum can therefore be used as an indicator for lignin (VSC lignin). Multiplication with the carbon content of the individual lignin oxidation products yields the amount of lignin carbon (C_{VSC}). The stable carbon isotope composition of the lignin monomers was determined in the extracts by gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS, Goñi and Eglinton, 1996; gas chromatograph Agilent Technologies HP 6890N Plus, USA, interface Combustion III, Finnigan Thermoquest, Germany and IRMS MAT 252, Finnigan, Germany). To volatilize lignin monomers in the extracts, the derivatization agent BSTFA/TMCS 99:1 was added 1:1 (vol.) prior to injection into the gas chromatograph. The shift in the isotopic composition due to the addition of trimethylsilyl carbon from BSTFA/TMCS 99:1 was corrected according to the mass balance equation given by Dignac et al. (2005) as described in Hofmann et al. (2009). For further details of the method please see Hofmann et al. (2009) and Heim and Schmidt (2007a). The proportions of old (C_3-derived) and new (C_4-derived) soil organic carbon and lignin were

calculated by adapting the formula established by Balesdent and Mariotti (1996), as suggested by Dignac et al. (2005) and Heim and Schmidt (2007a) (Eq. 1).

$$F_{\text{new}} = \frac{\Delta\ \delta^{13}C_{\text{soils}}}{\Delta\ \delta^{13}C_{\text{plants}}} \quad (1)$$

F_{new} is the fraction of new, C_4-derived SOC or lignin, $\Delta\delta^{13}C_{soils}$ is the difference between the delta values (‰V-PDB; determined by GC-C-IRMS) of SOC or lignin in the soil before and after the conversion to C_4-vegetation, and $\Delta\delta^{13}C_{plants}$ is the difference between the delta values of organic carbon or lignin extracted from the two different kinds of input vegetation (C_3- or C_4-derived). Quantities of C_4-derived SOC or lignin were calculated by multiplication of F_{new} with the SOC or lignin concentration determined by elemental analyses or GC-FID. C_3-derived SOC or lignin is the difference between total SOC or lignin and C_4-derived SOC or lignin. C_3-derived SOC or lignin can only decrease over the course of the experiment because new biomass input is exclusively from the new label, the C_4-vegetation. Therefore C_3-SOC or lignin can be used to describe the decay of old C_3-derived SOC or lignin, with "old" referring to the time before the experiment was started (1966).

2.4 Statistical analysis

In order to estimate change rates, we performed linear regressions of concentrations (lignin carbon C_{VSC}, soil organic carbon SOC) against time. The slope of the regression is the estimator of the change rate. We tested if the change rates were significantly different from zero with a t-test (p=0.05; df=4). To assess if nitrogen fertilization had an effect on C_{VSC} and SOC concentrations, we used a paired t-test for comparing measured concentrations of each date between the two treatments (p=0.05; df=8). Additionally, also with a t-test, we tested if mineral fertilization had a direct effect on the change rates (p=0.05; df=8).

3 Results

3.1 Total soil lignin carbon and soil organic carbon concentrations

Total lignin carbon (C_{VSC}) concentrations in soil ranged between 69 and 132 μg C_{VSC} g^{-1} soil (Fig. 1a) which corresponded to 9.6 and 17.0 mg C_{VSC} g^{-1} SOC (Table 1). Applying linear regressions, we found that C_{VSC} change rates were not significantly different from zero (Table 2: total C_{VSC}), suggesting that total lignin carbon concentrations remained constant during the experiment. Differences in total C_{VSC} concentrations between treatments were not consistent and not significant over time (Fig. 1a, Table 2: total C_{VSC}, fertilization effect). In addition, also the change rates were not significantly different between treatments (Table 2: total C_{VSC}, fertilization effect). From these results we can conclude that mineral fertilization had no effect on total soil lignin carbon concentrations in this field experiment.

Total SOC concentrations in this arable soil were relatively low, ranging between 7.0 and 8.7 mg SOC g^{-1} soil (Fig. 1d). Total SOC concentrations decreased slightly in both treatments over the course of the experiment (Fig. 1d, Table 2: total SOC). Similar to the results for total C_{VSC}, also for total SOC, no significant effects of mineral fertilization (Fig. 1d, Table 2: total SOC, fertilization effect) were observed during 36 years.

3.2 Accumulation of new, C_4-derived lignin carbon and C_4-derived OC in soil

New, C_4-derived (from maize biomass) lignin carbon (Fig. 1b) as well as C_4-derived SOC (Fig. 1e) accumulated slowly but significantly during the 36 years of the experiment (Table 2). This low accumulation rate was likely due to the relatively small amounts of aboveground plant residues returned to the soil with silage maize cropping. A similarly low accumulation rate of C_4-derived organic carbon was found in the silage maize experiment studied by Flessa et al. (2000). While we could show that there was no difference in the accumulation of new, C_4-derived lignin between the fertilization treatments (Table 2, fertilization effect C_4–C_{VSC}), we could also demonstrate that new C_4-SOC was significantly accumulated with mineral fertilization (Fig. 1e; Table 2, fertilization effect C_4-SOC). This result points out potential differences in the retention of new SOC and lignin. The enhanced accumulation of new, C_4-derived SOC was the only significant effect of mineral fertilization on C stocks in the soil of the studied field experiment.

3.3 Decay of old, C_3-derived lignin carbon and C_3-derived OC in soil

During the experiment, no fresh C_3-derived carbon from plant biomass was added to the soil. Thus the pre-existing C_3-derived SOC originating from the start of the experiment (age $\geq$36 years in the sampling year 2002) could be tracked for decay over time. Mineral fertilization had no effect on the decay of neither old, C_3-derived lignin nor C_3-SOC. Concentrations of old, C_3-derived lignin were similar between fertilization treatments (Fig. 1c; Table 1). The rate at which old, C_3-derived lignin carbon was lost from the soil was not affected by mineral fertilization (-1.8 ± 0.5 μg C_3–C_{VSC} g^{-1} soil a^{-1} in the non-fertilized treatment vs. -1.2 ± 0.6 μg C_3–C_{VSC} g^{-1} soil a^{-1} in the fertilized treatment, Table 2). Also old C_3-SOC concentrations seemed to be unaffected by the fertilization treatment (Fig. 1f). From the change rates (Table 2) it can be concluded that after 36 years on average about 44% of the initial lignin carbon concentration decomposed in comparison to only 24% of the initial SOC concentration, suggesting

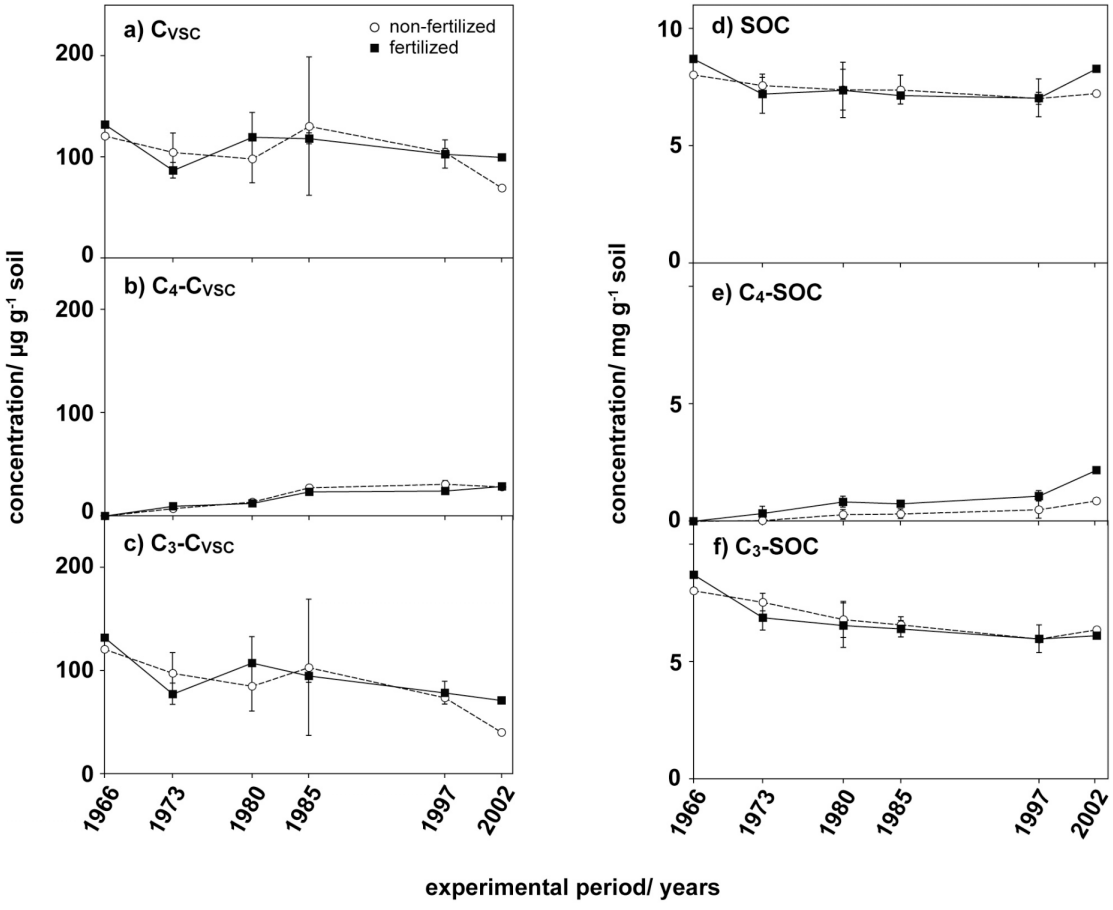

Fig. 1. Lignin carbon (C_{VSC}) and soil organic carbon (SOC) in archived soil of the continuous silage maize fertilization experiment at Cadriano (University of Bologna, Italy). Error bars denote the standard error of two field replicates. The starting year is represented by archived soil samples of the parallel continuous wheat plots.

that decay of old lignin was faster than decay of old bulk SOC.

3.4 Quality of new plant biomass input

Aboveground maize plant material (stover, i.e. stems, leaves, and husks) produced during the fertilization experiment had an average lignin carbon concentration of approximately 28 mg C_{VSC} g^{-1} plant dry weight (Table 3). This corresponded to an average of 63 mg C_{VSC} g^{-1} plant organic carbon (OC), as calculated from Table 3. Dignac et al. (2005) give similar concentrations for maize plant material, also measured after CuO oxidation: 55 mg C_{VSC} g^{-1} plant OC in the leaves (107 C_{VSC} g^{-1} plant OC in the stems and 103 C_{VSC} g^{-1} plant OC in the roots). While we found no significant differences in lignin concentrations between the treatments, OC concentrations were significantly higher in plant material from fertilized vs. non-fertilized plots (Table 3). In Fig. 2 we give quality parameters of aboveground maize biomass such as C/N and C_{VSC}/N ratios (Fig. 2a, b) for which increased values were found in non-fertilized maize plant material, indicating lower degradability. The quality of lignin, as assessed by ratios between lignin monomeric units, remained constant in fertilized plant material, while lignin in maize stover of non-fertilized plants showed relatively high variability (Fig. 2c, d).

4 Discussion

The fertilization treatment of the long-term field experiment was nitrogen and phosphorus (300 kg N ha^{-1}a^{-1}, 150 kg P ha^{-1}a^{-1}) in combination. All possible effects of the fertilization treatment on the decay of SOC and lignin (enhancing, retarding, no effect) would thus be due to both nutrients. However, the focus of our study was on the effect of nitrogen fertilization on lignin decay because nitrogen was suggested to reduce enzymatic lignin decay by Keyser et al. (1978), Fog et al. (1988), Carreiro et al. (2000), Hagedorn et al. (2003), Foereid et al. (2004). Phosphorus has not been

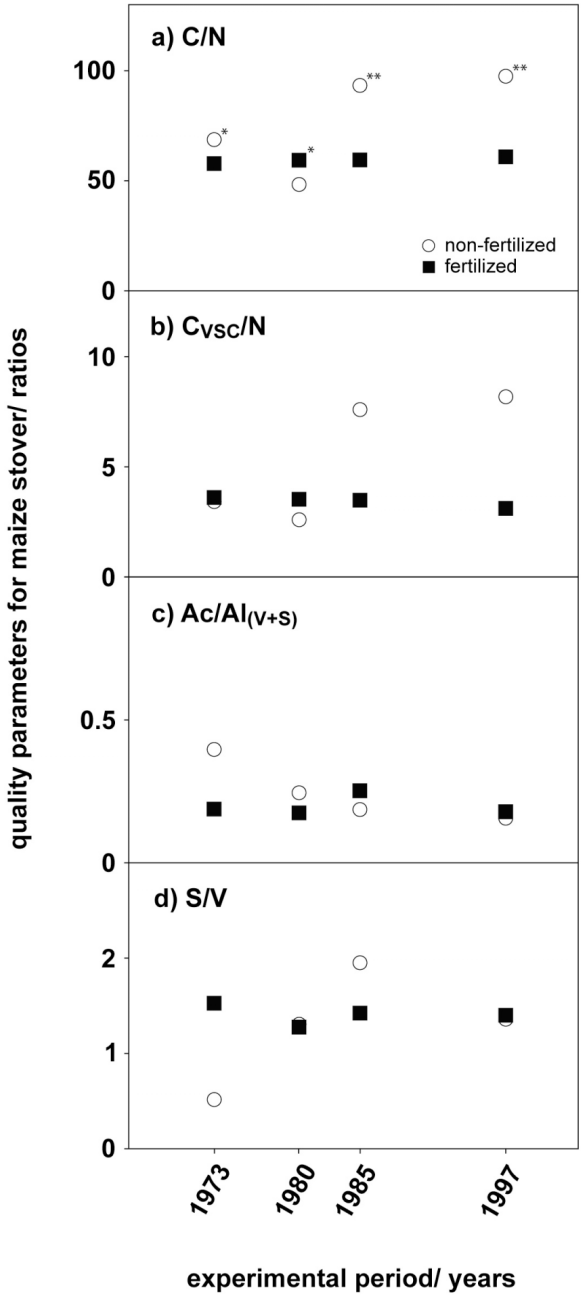

Fig. 2. Quality parameters for aboveground maize plant material (stover) of the continuous silage maize fertilization experiment at Cadriano (University of Bologna, Italy). Maize stover quality of non-fertilized plants showed relatively high variability, whereas the quality of fertilized plant material remained constant. Differences in means could statistically be proven for C to N ratios **(a)** for which n=3 analytical replicates existed. Levels of significance: $^{*}P < 0.05$ significant, $^{**}P < 0.01$ very significant. For parameters on the state of lignin decomposability **(b–d)** only one analytical replicate existed.

suggested in this context so far. We found only few studies on the effect of phosphorus on SOM decay. For an 18-year field experiment Han et al. (2006) show a smaller net loss of SOC when N+P were fertilized, instead of N alone. The field experiment of which we used archived soil samples was originally designed as a long-term maize cultivation experiment with a conventional agricultural fertilization treatment. We accepted the compromise that not only nitrogen but also phosphorus was fertilized because we were interested in this experiment for the unique long-term natural ^{13}C-labeling in combination with the fertilization treatment. Additionally, nitrogen fertilization alone in a long-term experiment might have induced phosphorus limitation both for maize plants as well as for microbial communities.

4.1 Does mineral fertilization reduce the decay of old lignin?

Our initial hypothesis that mineral fertilization reduces lignin decay for the studied long-term field experiment has to be rejected as the fertilization treatment did neither significantly affect lignin carbon concentrations (Fig. 1a), nor the decay of C_3-labeled old lignin carbon (Fig. 1c) in the soil over 36 years. Variations in the quality of plant lignin have been suggested to alter its degradability (Bahri et al., 2006). The absence of a fertilization effect on lignin decay is therefore in line with the constant quality of lignin inputs (Fig. 2c, d), which we assessed by comparing monomer abundances as introduced by Hedges and Mann (1979). Since we did not detect a fertilization effect on C_3-SOC decay during 36 years, we could not test if lignin might be a cause for retardation of C_3-SOC decay as it had been suggested by Fog (1988). We suggest that in those experiments that found retarding effects on SOC decay by mineral fertilization, lignin concentrations should be measured before such an effect can be attributed to lignin. Alternatively, as done by Keeler et al. (2009), studying the activity of lignin-degrading enzymes (phenol oxidase, peroxidase) might provide the results independently from analysis of lignin concentrations.

4.2 Why do we not find fertilization effects on decay in this field experiment?

One reason for not detecting significant differences between the mineral fertilization treatments might be the fact that field conditions were influenced by atmospheric nitrogen deposition (Holland et al., 2005). With field conditions, no total absence of nitrogen input can be achieved in the way it could be simulated in laboratory or semi-field experiments as conducted by Keyser et al. (1978), Magill and Aber (1998), Carreiro et al. (2000), Hagedorn et al. (2003) and Foereid et al. (2004). The difference between nitrogen levels might not have been large enough in the studied field experiment to induce significant differences in decay dynamics. This is supported by the findings of Henriksen

Table 1. Lignin carbon concentrations in SOC, carbon to nitrogen ratios (C/N) and lignin carbon to nitrogen ratios (C_{VSC}/N) of soil samples from two nitrogen fertilization treatments of the Cadriano continuous silage maize fertilization experiment (University of Bologna, Italy). Soil samples are from the ploughing horizon (0–ca. 45 cm), sampling depth was 25 cm in 1973, 1980, 1985 and 40 cm in 1997 and 2002. Results are given as the mean with the standard error of two field replicates.

Treatment	Crop	Sample year	Lignin carbon (C_{VSC}) /mg g^{-1} SOC	C_4-lignin carbon (C_4-C_{VSC})	C_3-lignin carbon (C_3-C_{VSC})	C/N	C_{VSC}/N
non-fertilized	Wheat	1973	15.0±n.a.[a]	0	15.0±n.a.[a]	7.1±n.a.[a]	0.11±n.a.[a]
non-fertilized	Maize	1973	13.7±1.9	1.0±0.2	12.8±2.1	7.1±0.4	0.10±0.02
		1980	13.1±1.7	1.8±0.3	11.3±2.0	8.6±1.0	0.11±0.03
		1985	17.0±7.8	3.7±0.0	13.3±7.8	8.0±0.9	0.14±0.07
		1997	14.9±1.1	4.4±0.7	10.5±0.4	7.4±0.3	0.11±0.00
		2002	9.6±n.a.[a]	3.9±n.a.[a]	5.5±n.a.[a]	7.7±n.a.[a]	0.07±n.a.[a]
Change rate[b]/a^{-1}			−0.1±0.1	0.1±0.0	−0.2±0.1	0.1±0.2	0.00±0.01
P change rate[c]			0.376 n.s.	0.007 **	0.028 *	0.677 n.s.	0.553 n.s.
fertilized	Wheat	1973	15.2±n.a.[a]	0	15.2±n.a.[a]	8.1±n.a.[a]	0.12±n.a.[a]
fertilized	Maize	1973	12.3±2.5	1.3±0.2	11.0±2.7	8.0±1.3	0.10±0.01
		1980	16.1±0.7	1.7±0.4	14.4±1.1	8.3±1.6	0.13±0.03
		1985	16.6±1.6	3.3±0.0	13.4±1.6	7.5±0.5	0.12±0.01
		1997	14.6±0.3	3.5±0.0	11.1±0.3	8.1±1.2	0.12±0.02
		2002	12.0±n.a.[a]	3.5±n.a.[a]	8.6±n.a.[a]	9.6±n.a.[a]	0.12±n.a.[a]
Change rate[b]/a^{-1}			0.0±0.1	0.1±0.0	−0.1±0.1	0.3±0.2	0.00±0.00
P change rate[c]			0.607 n.s.	0.008 **	0.088 n.s.	0.259 n.s.	0.906 n.s.
Fertilization effect:							
P paired t-test[d]			0.457 n.s.	0.191 n.s.	0.312 n.s.	0.178 n.s.	0.256 n.s.
P t-test for change rates[e]			0.692 n.s.	0.435 n.s.	0.428 n.s.	0.542 n.s.	0.553 n.s.

[a] No replicate sample available.
[b] Slope of linear regression.
[c] Probability of error. Levels of significance: $P>0.05$ not significant n.s., $P<0.05$ significant *, $P<0.01$ very significant **, $P<0.001$ highly significant ***.
[d] Pairs are the results of treatments for individual sample years; tests the effect of mineral fertilization on the variable.
[e] Tests if mineral fertilization had an effect on the change rates.

and Breland (1999) who showed that mineralization might be retarded only at rather high nitrogen concentrations. According to the model proposed by Schimel and Weintraub (2003), it requires low amounts of N to maintain a maximal rate of microbial decomposition. These results are supported by Keeler et al. (2009), who, in their recent paper on the topic of microbial enzyme activity and OC decomposition, did not find an effect of nitrogen fertilization on lignin degrading enzyme activity in grassland and forest ecosystem field plots. In summary, it becomes clear that, although lab experiments are essential for studying mechanistic relationships, they should be complemented by field experiments. In addition to direct, mechanistic effects which might be predictable from lab studies, the relevant actual effect under field conditions on the total agro-ecosystem is potentially additionally controlled by feedback effects such as a preferential microbial decomposition of fresh maize biomass input instead of older soil organic carbon moieties.

A second reason for not detecting significant differences might be the fact that in this long-term field experiment the fertilization treatment was nitrogen and phosphorus (300 kg N $ha^{-1}a^{-1}$, 150 kg P $ha^{-1}a^{-1}$) in combination, and not nitrogen alone as in previous laboratory or semi-field experiments (Keyser et al. (1978), Magill and Aber (1998), Carreiro et al. (2000), Hagedorn et al. (2003) and Foereid et al. (2004)). Nitrogen fertilization alone in a long-term experiment might have induced phosphorus limitation both for maize plants as well as for microbial communities, which would have been an undesirable side effect and is therefore avoided in conventional agricultural practice. Also the

Table 2. Results of linear regression for organic carbon and lignin carbon concentrations in soil samples of the Cadriano continuous silage maize fertilization experiment (University of Bologna, Italy).

Treatment	total SOC	C_4-SOC	C_3-SOC	total C_{VSC}	C_4-C_{VSC}	C_3-C_{VSC}
non-fertilized						
Change rate[a]/μg g^{-1} soil a^{-1}	−22.2±6.0	22.6±3.4	−51.8±11.2	−0.9±0.6	0.9±0.2	−1.8±0.5
P change rate[b]	0.021 *	0.003 **	0.010 *	0.205 n.s.	0.008 **	0.018 *
fertilized						
Change rate[a]/μg g^{-1} soil a^{-1}	−9.9±25.1	50.9±10.7	−60.7±21.0	−0.5±0.6	0.8±0.1	−1.2±0.6
P change rate[b]	0.715 n.s.	0.009 **	0.045 *	0.452 n.s.	0.004 **	0.106 n.s.
Fertilization effect:						
P paired t-test[c]	0.443 n.s.	0.031 *	0.574 n.s.	0.531 n.s.	0.302 n.s.	0.416 n.s.
P t-test for change rates[d]	0.646 n.s.	0.036 *	0.719 n.s.	0.588 n.s.	0.644 n.s.	0.452 n.s.

[a] Slope of linear regression.
[b] Probability of error. Levels of significance: $P>0.05$ not significant n.s., $P<0.05$ significant *, $P<0.01$ very significant **, $P<0.001$ highly significant ***.
[c] Tests the effect of mineral fertilization on the variable.
[d] Tests the effect of mineral fertilization on the change rates.

Table 3. Total organic carbon and lignin carbon concentrations in aboveground plant material from two nitrogen fertilization treatments of the Cadriano continuous silage maize fertilization experiment (University of Bologna, Italy) and corresponding yield data for maize grain and stover (stem and leaves). Results are given as the mean with the standard error of two field replicates, if available.

Treatment	Crop	Sample year	Organic carbon (OC) /mg g^{-1} dry weight	Lignin carbon (C_{VSC}) /mg g^{-1} dry weight	/mg g^{-1} OC	Grain yield /t dry weight ha^{-1}	Stover yield /t dry weight ha^{-1}
non-fertilized	Wheat	1980	389.0±2.0	34.0±0.6	87.4±10.1	n.a.[a]	n.a.[a]
non-fertilized	Maize	1973	406.7±7.9	20.3±n.a.[a]	49.9±n.a.[a]	3.2±n.a.[a]	3.1±n.a.[a]
		1980	379.0±0.4	20.4±n.a.[a]	53.9±n.a.[a]	0.8±n.a.[a]	1.9± n.a.[a]
		1985	397.1±3.2	32.4±n.a.[a]	81.5±n.a.[a]	2.4±n.a.[a]	4.3±n.a.[a]
		1997	405.1±2.2	34.0±n.a.[a]	84.0±n.a.[a]	0.8±n.a.[a]	1.5±n.a.[a]
		2002	n.a.[a]	33.1±3.4	n.a.[a]	2.5±n.a.[a]	2.3±n.a.[a]
fertilized	Wheat	1980	397.8±6.8	37.7±0.1	94.8±19.6	n.d.[a]	n.d.[a]
fertilized	Maize	1973	421.4±0.1	26.3±n.a.[a]	62.5±n.a.[a]	7.4±n.a.[a]	4.3±n.a.[a]
		1980	415.7±2.8	24.7±n.a.[a]	59.5±n.a.[a]	6.1±n.a.[a]	6.5±n.a.[a]
		1985	424.4±1.3	24.9±n.a.[a]	58.6±n.a.[a]	8.5±n.a.[a]	9.5±n.a.[a]
		1997	431.0±1.5	22.1±n.a.[a]	51.2±n.a.[a]	7.7±n.a.[a]	5.0±n.a.[a]
		2002	n.a.[a]	40.3±3.3	n.a.[a]	6.6±n.a.[a]	6.0±n.a.[a]
Fertilization effect:							
P paired t-test[b]			0.010 **	0.926 n.s.	0.455 n.s.	0.001 ***	0.006 **

[a] No sample or no replicate sample available.
[b] Pairs are the results of treatments for individual sample years; tests the effect of nitrogen fertilization on the variable. P Probability of error. Levels of significance: $P>0.05$ not significant n.s., $P<0.05$ significant *, $P<0.01$ very significant **, $P<0.001$ highly significant ***.

long-term experiment discussed by Khan et al. (2007) received a full conventional fertilization treatment and was not able to observe a retarding effect of fertilization on SOC decomposition as previous "N-only" experiments had done. This raises the question whether previously observed retarding effects of N on decomposition could have been indirect effects by inducing P limitation in decomposer communities, but this question cannot be answered with our study.

4.3 Estimates of the belowground input of new, maize-derived organic carbon and lignin

According to the review by Amos and Walters (2006) we can assume a net belowground carbon deposition (root biomass and rhizodeposition) of 29 ±13% of shoot (= stover) biomass carbon for maize at physiological maturity. From the data on stover yield in Table 3 we can therefore estimate the belowground carbon deposition was $757 \pm 344\,\mathrm{kg\,OC\,ha^{-1}a^{-1}}$ in the fertilized plots. Taking into account that root/shoot ratios increase by 41.6 ±8.6% under nitrogen deficiency (Amos and Walters, 2006), net belowground carbon deposition in the non-fertilized plots was $421 \pm 242\,\mathrm{g\,OC\,ha^{-1}a^{-1}}$ (calculated from Table 3). With a lignin concentration of 10.3% in organic carbon of maize roots (Dignac et al. 2005) as a basis, the belowground lignin carbon deposition from belowground maize biomass could be estimated as $79\,\mathrm{kg\,C_{VSC}\,ha^{-1}\,a^{-1}}$ in the fertilized plots versus $44\,\mathrm{kg\,C_{VSC}\,ha^{-1}\,a^{-1}}$ in the non-fertilized plots (calculated from Table 3). From these calculations, we suggest a doubled net belowground carbon and lignin input in fertilized plots in comparison to non-fertilized plots. Information on belowground carbon input is important because in this experiment the maize crop was harvested as silage (all aboveground plant parts were removed from the field) and only roots and stubbles remained as effective C_4-labeled maize biomass input.

4.4 Mineral fertilization might have enhanced the decay of new lignin

The increased biomass production in the fertilized treatment can be related to the actual SOC accumulation in the soil, so that conclusions on decay of fresh biomass input can be drawn. While we estimated that the input of total belowground maize biomass carbon approximately doubled in the fertilized plots (Sect. 4.3), we also measured a doubled actual accumulation of C_4-derived SOC (Table 2, change rates, C_4-SOC; Fig. 1e). This result suggests that the decay of fresh maize-derived organic carbon was similar in the two fertilization treatments. In contrast, C_4-lignin accumulation (Fig. 1b, Table 2, C_4–C_{VSC}) was not matching the estimated doubled input (Sect. 4.3) under mineral fertilization, proposing that decay of lignin from fresh biomass input might have been enhanced in fertilized plots. This interpretation relies on the assumption that lignin concentrations in root derived organic matter were not affected by fertilization, similarly to what we could show for aboveground plant material (Table 3). We propose that decay of new lignin might be enhanced by mineral fertilization in contrast to no effect on decay of old lignin. This however would contrast studies stating that it is rather the limitation of nitrogen that increases litter decomposition (Craine et al., 2007). To explain the opposing findings it would be necessary to analyse lignin concentrations in experiments where clear fertilization effects (either enhancement or retardation) on SOC were found. In those cases lignin concentrations might help to explain the effect on total SOC.

4.5 Lignin decay was faster than SOC decay

Our results support earlier evidence that lignin might decompose faster than bulk SOC (Kiem and Kögel-Knabner, 2003). With 56% of the initial C_3-lignin carbon and 76% of the initial C_3-SOC still measurable in the soil after 36 years, we found an overall slow mineralization of C_3-SOC and C_3-lignin in the studied long-term field experiment. This result is in accordance with our finding in a previous study where we proposed that about two thirds of the initial C_3-lignin was stabilized during 18 years (Askov continuous silage maize, Hofmann et al., 2009). Similarly, 64% of C_3-SOC was detected after 23 years in another arable soil experiment (Boigneville, Heim and Schmidt, 2007a). A possible explanation for the overall slow mineralization of lignin and SOC could be an intensive cropping of the soils before the experiments were initiated. Intensive cropping, including aeration of the soil with ploughing, could have resulted in mineralization of most old C_3-carbon, leaving only carbon that was already stabilized. This suggestion is supported by the result from a field experiment where only 28% of initial C_3-lignin was stabilized over the duration of 23 years (Rotthalmünster, Heim and Schmidt, 2007b). The fast mineralization found in this study might be related to the fact that the arable soil had been established on former cultivated grassland, and thus might not have been in equilibrium for carbon stocks in contrast to arable soils that have been intensively worked for decades.

5 Conclusions

In a natural agro-ecosystem, decay of old, stabilized lignin was less sensitive to mineral fertilization than previously suggested. Mineral fertilization neither retarded nor enhanced the decay of old, C_3-labeled SOC or lignin over a period of 36 years.

Mineral fertilization might have had an effect on new, non-stabilized lignin carbon. For fresh C_4-labeled biomass there were indications for SOC and lignin decay being decoupled. Decay of C_4-labeled lignin from fresh biomass might have been enhanced by mineral fertilization, whereas decay of C_4-SOC was not. Mineral fertilization might thus have neither

an effect on already stabilized old SOC or lignin nor on new SOC, but might affect new, non-stabilized lignin.

5.1 Author contributions

The study was proposed and supervised by A. Heim, M. W. I. Schmidt and A. Miltner. P. Gioacchini provided the samples and conducted the EA-IRMS measurements. M. Gehre supervised the GC-C-IRMS analyses. Lignin extraction, GC-FID, GC-C-IRMS, data analysis and paper writing was completed by A. Hofmann with contributions of all co-authors.

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Manuscript III

^{13}C-labelled lignin and SOC in physical soil fractions of an arable soil after 18 years - distribution and stock changes

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Abstract

Density and particle size fractionation studies of soils from different land uses suggest that lignin in soil is mainly comprised in the coarse, light material (particulate organic matter). In a previous study we found that more than 60 mass-% of the initial lignin concentrations had been preserved in an arable soil over the time period of 18 years. How could this lignin have been protected from decomposition? The first objective of this study was to measure the distribution of ^{13}C labeled lignin in physical soil fractions in order to find the soil fractions that are important for holding stocks of lignin in the studied soil. The second objective was then to identify those soil fractions that show smallest losses of ^{13}C labeled lignin over time. The fractions might be related to possible lignin stabilization mechanisms and can be used to explain how lignin is preserved in soil over several decades. In our study we combined the methods of physical soil fractionation with the compound specific analysis of lignin (CuO oxidation) and natural ^{13}C abundance labeling. For the physical fractionation we used archived soil samples from years 0 and 18 of the continuous maize experiment at Askov, DK. Important stocks of lignin and SOC were comprised in the coarse heavy fraction (250-2000 μm, density > 2.25 g cm^{-3}). About 1/3 of the total lignin and SOC was in this fraction at the start of the experiment. Over the period of 18 years, smallest losses of lignin were found in the coarse light fraction and in the silt size fraction. Only 10 and 23 mass-% of the initial C_3-derived lignin were decomposed in these fractions. Most of the organic carbon within the coarse heavy fraction seemed to be stabilized by aggregation. The particulate organic matter in the light fraction might have been preserved because of spatial inaccessibility due to encrustation with minerals. The results point out that lignin might, to a certain extent, be protected from decomposition by mineral particles.

Introduction

Physical fractionation separates bulk soil according to particle sizes or/and density of its mineral and organic components (Elliott & Cambardella, 1991; 1992; von Lützow et al., 2007), which is acknowledged as a “pretreatment for further differentiation of functional fractions” (Kögel-Knabner et al., 2008). To gain primary particles, the soil is dispersed completely by ultrasonification (Christensen, 1985; Christensen, 1992; Amelung & Zech, 1999; Schmidt et al., 1999). When the ultrasonification step is omitted however, water-stable aggregates instead of primary particles can be recovered from the soil (Six et al., 1998). One important aim of fractionation is to find soil components with special traits or functions that help explain mechanisms and processes in soils. These fraction could represent measurable pools for soil organic carbon modeling (Sohi et al., 2001; Zimmermann et al., 2007) and might be related to stabilization mechanisms (von Lützow et al., 2007).

Lignin is a component of SOC with a complex phenolic structure. It originates from plant cell walls and is only synthesized by vascular plants (Pearl, 1967; Hatakka, 2001). Any lignin in soils can thus only derive from plant residues and cannot be re-synthesized by soil microorganisms like carbohydrates or lipids could. This fact allows for the direct study of quantitative decay by measuring concentrations of labeled lignin in soil over long time periods (Dignac et al., 2005; Heim & Schmidt, 2007a; Heim & Schmidt, 2007b; Hofmann et al., 2009a; Hofmann et al., 2009b). These experiments on lignin decay in soil all rely on natural ^{13}C abundance labeling after vegetation conversion from C_3 crops (e.g. wheat, barley) to C_4 crops (e.g. maize) (Balesdent et al., 1987). The aim of the studies on lignin decomposition is to explain the decomposition dynamics and provide turnover times. Dignac et al. (2005) and Heim and Schmidt (2007a) proposed lignin turnover times of a few years up to several decades. These calculations of turnover times however are based on the assumption of a single pool with exponential decay, which underestimates a potentially present slow pool. As it was previously demonstrated by Lobe et al. (2002), Rasse et al. (2006) could model lignin decay with two pools, a fast (turnover time < 1 year) and a slow pool (20 years). In two long-term field experiments it could be shown that the amount of lignin remaining in the soil after 23 years (Heim & Schmidt, 2007a) and after 18 years (Hofmann et al., 2009a) ranged from one fourth to two thirds of the initial lignin.

What is known about lignin in soil fractions? Lignin concentrations decrease with increasing density and decreasing particle size (Guggenberger et al., 1994; Amelung et al., 1999; Sollins et al., 2006), suggesting that most lignin is in coarse, light material (particulate organic matter POM). It is important to note that concentrations in fractions can only provide information about the fraction's composition. Information about the distribution and importance of fractions with regard to e.g. lignin can be deduced only when the concentrations in fractions are related to the mass of fractions relative to the bulk soil. Even with a high concentration of lignin a fraction could represent only a small portion of the lignin in the bulk soil if the overall mass of this fraction is small. Therefore complete mass distributions should be calculated whenever possible for evaluating the importance of the individual fractions (Christensen, 1996b). Only one study so far used stable isotopes to evaluate lignin turnover in soil fractions: Heim & Schmidt (2007b) In measured lignin in particle size fractions to show that the silt fraction is important for stabilization of old lignin.
In this study, physical fractionation was achieved by a combined aggregate size and density fractionation as adapted from Six et al. (1998). We used soil samples of year 0 and 18 from the Askov continuous maize experiment (Kristiansen et al., 2005; Hofmann et al., 2009a), which includes a ^{13}C natural abundance label. The main research questions of our study were:
(i) How are ^{13}C labelled lignin and SOC distributed over physical soil fractions? Data on distribution can show in which soil fractions lignin and SOC are preferentially accumulated.
(ii) How did stocks of ^{13}C-labelled lignin and SOC change over time (18 years)?
Data on stock changes can show if lignin and SOC is preferentially degraded in one fraction compared to other fractions.

Material and methods

Soil samples of the continuous maize experiment at Askov, DK

For the physical fractionation we used archived soil samples (dried, sieved to 2 mm) from 1988 (year 0) and 2006 (year 18) of the continuous maize experiment at Askov, Denmark (55°28'N, 09°07'E; mean annual temperature 7.7°C; mean annual precipitation 862 mm; Christensen, 1997). The experiment includes a C_3- to C_4-vegetation conversion, which naturally labeled soil organic carbon with a higher abundance of ^{13}C isotopes (Balesdent et al., 1987) starting in 1988. We used soil samples from the part of the experiment where biomass input (800 g dry matter m^{-2}) was increased by input from aboveground maize plant material, which simulated incorporation of maize stover into the soil. Soil samples were from the ploughed horizon, 0-20 cm. The soil has a sandy loam texture (65% sand, 21% silt, 14 % clay), a neutral pH (6.4) and a bulk density of 1.12 g cm^{-3} (Kristiansen et al., 2005).

Combined aggregate size and density fractionation

The fractionation protocol was adapted from Six et al. (1998) with density cutoffs adapted from Sollins et al. (2006) and is shown in Figure 1. As suggested by Six et al. (1998), no ultrasonic dispersion was included during the fractionation, with the aim to preserve water stable aggregates. Aggregate size fractionation was adapted by using a stack of test sieves (250 μm, 20 μm) on a sieve shaker (Retsch AS 200 control, Haan, Germany) instead of manual sieving (Six et al., 1998). We successively wet-sieved 5 x 20 g of soil sample for two minutes at a gentle sieve plate acceleration of 3.0 *g* (oscillation amplitude 0.5 mm) after submerging the soil for 5 minutes in deionized water. Soil particles 0.45 - 20 μm were vacuum-filtered from the water after wet sieving (cellulose acetate membrane filters 0.45 μm, Whatman GmbH, Dassel, Germany). Particle sizes 2-20 μm and <2 μm were separated by sedimentation in Atterberg cylinders. DOC (<0.45 μm) was analyzed for the carbon mass balance. Using the described procedure, four classes of size fractions could be gained: 250- 2000 μm (macroaggregates), 20- 250 μm (microaggregates), 2-20 μm (fine silt-size aggregates), <2 μm (clay-size aggregates). Of these size fractions only the two largest yielded enough mass so that they could be used for subsequent density fractionation with sodium polytungstate solution (Fluka, Steinheim, Germany). We used density cutoffs at 1.85 g cm^{-3} to gain particulate organic matter (light fraction) and at 2.25 g cm^{-3} to gain mostly mineral matter (heavy fraction). Using the mixing-model by Chenu & Plante (2006) for density calculations of organo-mineral associations (assumptions: 2.6 g cm^{-3} for a mixed mineralogy clay fraction and 1.4 g cm^{-3} for organic matter), the density cutoff 1.85 g cm^{-3} would isolate a light fraction with ca 50 %

OM or 29 % C content and thus allows for minerals to be bound to the particulate organic matter. The density cutoff 2.25 g cm^{-3} on the other hand would allow for a heavy fraction with low OM contents of <20 % or <12 % C respectively. In order to estimate the amount of sand particles vs. aggregates in the coarse, heavy fraction (2H), we used the density cutoff 2.65 g cm^{-3} (density of quartz) on a sub-sample of the fraction (no ultrasonification treatment) as a supplementary density fractionation not included in the main fractionation scheme.

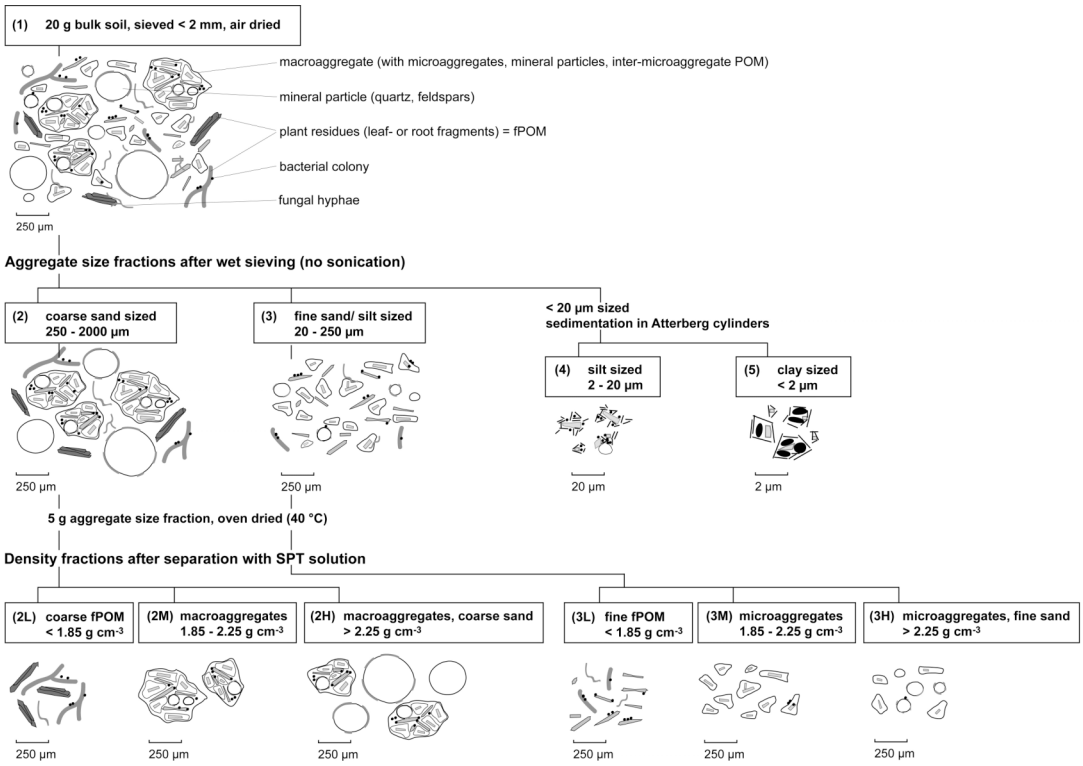

Figure 1 Fractionation scheme for combined aggregate size and density fractionation as adapted from Six et al. (1998). Density cutoffs adapted from Sollins et al. (2006). Size definition of micro- and macroaggregates according to Jastrow and Miller (1998). POM = particulate organic matter, fPOM = free POM, SPT = sodium polytungstate.

SOC analyses

All samples were analyzed in two replicates per fraction with an elemental analyzer (NA2500, CE-Instruments, Rodano, Milano, Italy) coupled to an isotope mass spectrometer (Delta plus, Finnigan MAT, Bremen, Germany; Interface Conflo III, Thermo Electron Cooperation, Bremen, Germany) for determining $d^{13}C$ of SOC. Carbon and nitrogen concentrations were measured with a thermal conductivity detector (TCD). The analyses were conducted by the Centre for Stable Isotope Research and Analysis, Göttingen, Germany. Average analytical precision (SE in % of mean, N = 28) was 6.4% for carbon (TCD), 4.0% for nitrogen (TCD) and 0.9% for $d^{13}C$ V-PDB.

Lignin analyses

Lignin monomers were extracted from soil fractions by alkaline cupric oxide oxidation in a microwave digestion system (Goñi & Montgomery, 2000 as adapted by Heim & Schmidt, 2007a). In the extracts, the oxidation products vanillyl, syringyl and cinnamyl phenols were quantified and their sum used as an indicator of lignin (VSC). Lignin carbon (C_{VSC}) was calculated from the molecular formulae of the individual lignin oxidation products (for monomers see Heim & Schmidt, 2007a), which contain between 55 and 65 mass-% C. Quantification of VSC was conducted by gas chromatography coupled to a flame ionization detector (GC-FID; HP 6890N Plus, Agilent Technologies, USA) as described in Hofmann et al. (2009a). In order to volatilize lignin monomers from extracts (dissolved in ethyl acetate), BSTFA/TMCS 99:1 derivatization agent was added 1:1 (vol.). Quantification was achieved using calibration curves of external lignin monomer standards. To correct for losses during sample preparation, cinnamic acid and ethyl vanillin were used as internal standards (Heim & Schmidt, 2007a). The average relative standard error for lignin analysis at the GC-FID was 5 %. Compound-specific isotope analysis for lignin monomers (Goñi & Eglinton, 1996) was conducted in duplicate for each soil extract using gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS; gas chromatograph HP 6890N Plus, Agilent Technologies, USA, interface Combustion III, Finnigan-Thermoquest, Germany and isotope ratio mass spectrometer MAT 252, Finnigan). For GC-C-IRMS measurements we used the alkanes n-20 and n-24 as internal standards (Heim & Schmidt, 2007a). Correction for the shift in the isotopic composition by adding trimethylsilyl C during derivatization (BSTFA/TMCS 99:1) was conducted according to the mass balance equation by Dignac et al. (2005) as described in Hofmann et al. (2009a).

Fractions of old lignin and SOC

The fraction of old, C_3-derived lignin carbon (C_3-C_{VSC}) within total lignin carbon (C_{VSC}) or of old, C_3-derived SOC within total SOC was calculated by adapting the formula for SOC established by Balesdent & Mariotti (1996), as proposed by Dignac et al. (2005) and Heim & Schmidt (2007a, Equation 1).

$$F_{old\,C} = 1 - \left(\frac{\Delta\,\delta^{13}C_{soils}}{\Delta\,\delta^{13}C_{plants}} \right) \quad (1)$$

$F_{old\,C}$ is the fraction of old, C_3-derived lignin carbon or C_3-derived SOC, $\Delta\,\delta^{13}C_{soils}$ is the difference between the delta values (‰ V-PDB; determined by GC-C-IRMS) of lignin or OC extracted from soil before and after the conversion and $\Delta\,\delta^{13}C_{plants}$ is the difference between the delta values of lignin or OC extracted from the input vegetation. Quantities of C_3-derived lignin or SOC were calculated by multiplication of $F_{old\,C}$ with the lignin or SOC concentration determined by GC-FID or EA.

XRD

The fractions were analyzed for their mineralogical composition using x-ray diffractometry (XRD; Cu Ka, 40kV, 40 mA, variable automatic divergence slit 20 mm, soller slit 4°, secondary graphite monochromator, detector NaI) at a Bragg-Brentano 2Theta-diffractometer (Bruker AXS D8 Advance, Diffrac, Karlsruhe, Germany). Analyses were conducted at the Institute for Geotechnical Engineering, ETH Zurich, Switzerland. Mineralogical concentrations were quantified using Rietveld analysis (software Rayflex Autoquan, General Electric, Ahrensberg, Germany).

Surface area analyses

For measuring specific surface areas of the isolated fractions we conducted a 5-point BET-N_2 surface area analysis (Gemini surface area analyzer, Micromeritics, Norcross GA, USA). The method employs a flowing-gas technique with the analysis gas N_2 flowing into two tubes (one sample and one empty balance tube) simultaneously.

Scanning electron microscopy

To visualize the fractions we used scanning electron microscopy (SEM; Zeiss SUPRA 50VP, Oberkochen, Germany), which allows to study the surface, or near surface, structure of a sample (Goodhew et al., 2001). For imaging the secondary electron detector (SE) was used. For element analysis an energy-dispersive X-ray-detector (EDS; EDAX Genesis Apex 4, Mahwah NJ, USA) was used. The electron source was from field emission, the electrons were accelerated to an energy of 2 keV for imaging (SE) and 10 keV (EDX). To prevent charging effects at the surface the samples were coated with a thin (~10-15 nm) conducting layer of carbon by sputter coating.

Estimation of organic carbon content in fractions explained by clay minerals

The carbon content of the clay-size fraction measured by EA and the content of clay minerals determined by XRD for each fraction was used for estimating the maximal potential proportion of carbon retained by the clay minerals of each soil fraction (Equation 2). We assumed that (i) all the carbon in the clay-size fraction was associated to clay minerals and that (ii) clay minerals in other fractions adsorbed the same amounts of carbon than the clay minerals in the clay-size fraction.

$$F_{\text{C retained by clay}} = \frac{(\text{mg clay minerals in fractions g}^{-1}\text{soil} * \text{mg C g}^{-1}\text{clay in} < 2\ \mu\text{m})/1000}{\text{mg C g}^{-1}\text{soil}} \quad (2)$$

This estimation provides the opportunity to test how important adsorption to clay minerals might be in each fraction.

Results

Distribution of 13C labeled lignin and SOC over physical soil fractions in year 0 and after 18 years

At the start of the experiment (year 0) lignin and SOC was predominant in the coarse fraction, in material with a density of > 2.25 g cm^{-3} (Figure 2a, c). After 18 years the coarse fraction still stored the largest stock of SOC, but the mass distribution had changed for lignin. Labeled C_3-derived lignin remained mainly in the light fraction (< 1.85 g cm^{-3}) and in the coarse heavy fraction (Figure 2b), making those the most important fractions for stocks of old lignin in year 18. The SOC on the other hand was still mainly stored in the coarse heavy fraction after 18 years (Figure 2d).

In comparison to the coarse heavy fraction, other fractions, in particular the light fraction and the silt or clay size fractions, constituted much smaller stocks. Overall, the same fractions that held least SOC also contained least lignin. However, the coarse light fraction seemed to be of relative more importance for lignin than for SOC. In year 0, the coarse light fraction stored 8 mass-% of total lignin in comparison to only 2 mass-% of total SOC (Figure 2a,c). In year 18 the difference was even larger with 33 mass-% of lignin in the coarse fraction compared to only 1 mass-% of total SOC (Figure 2b,d). Overall, in year 18 the coarse light and heavy fractions were the most important fractions for stocks of old lignin.

Decay of C_3-derived lignin and SOC in physical soil fractions within 18 years

C_3-derived lignin decomposed significantly in most fractions, exceptions were the coarse light fraction as well as the silt fraction, where only 10 and 23 mass-% of the initial C_3-derived lignin were decomposed (Figure 3). Decay of labeled SOC was overall smaller than decay of lignin except in fraction 2L (Figure 3).

Similar to the results for lignin, SOC decay was low in silt and clay. Differently from lignin decay, SOC decay was also especially low in heavy fractions (Figure 3).

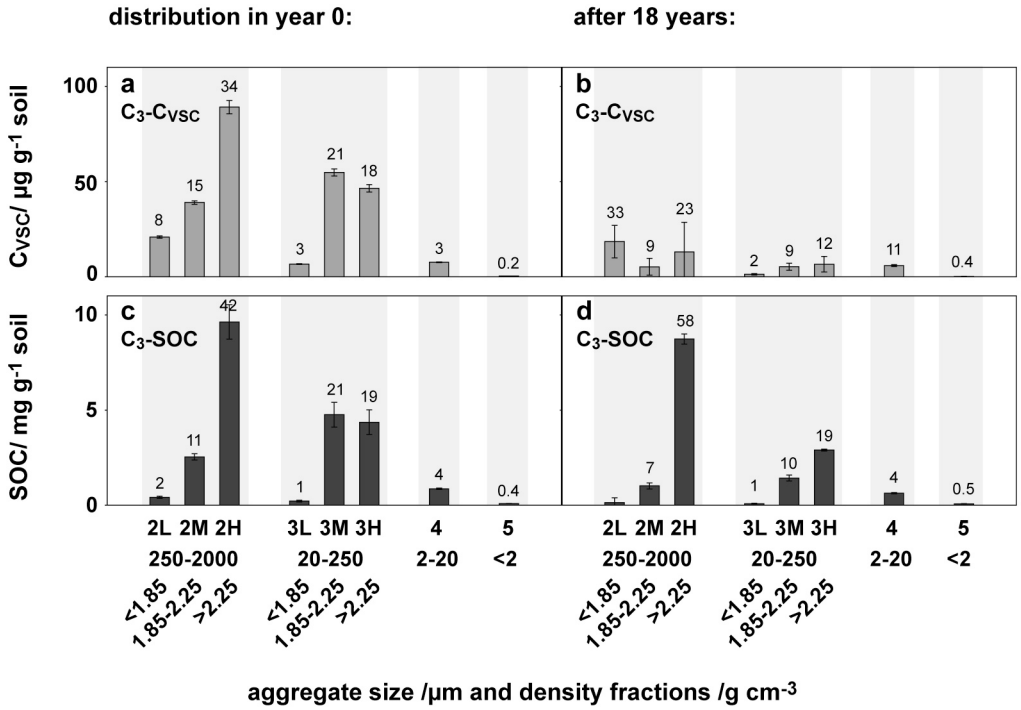

Figure 2 Distribution of C_3-lignin and C_3-SOC in the soil in year 0 and year 18, numbers above columns denote distribution in mass-% of total C_3-C_{VSC} or C_3-SOC concentration in bulk soil. **2a, c** start of the experiment, **2b, d** distribution after 18 years.

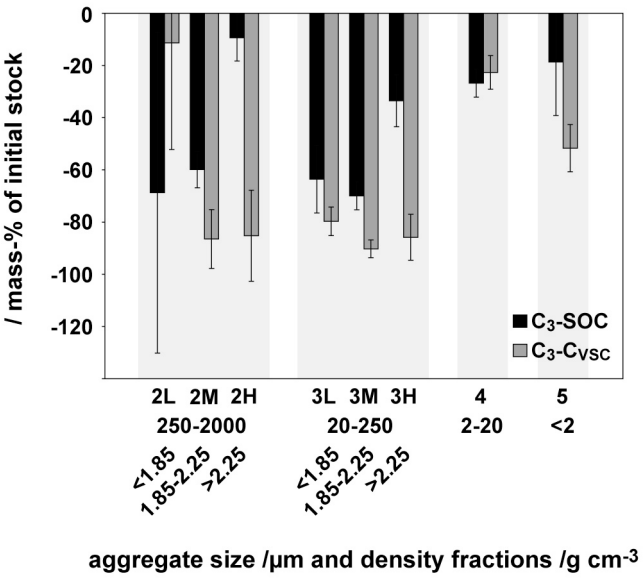

Figure 3 Relative changes in percent of initial C_3-lignin and C_3-SOC stocks within 18 years. Error bars represent error propagation from analytical replicates (n = 2). Large error bars with coarse light fraction (2L, 250 – 2000 μm, r < 1.85 g cm^{-3}) are due to heterogeneity of the fraction.

Lignin quality in the fractions

Figure 4 depicts trends for lignin degradation in soil. We found increasing acid to aldehyde ratios ($Ac/Al_{(V+S)}$) with increasing density (Figure 4a) which indicates advanced oxidation in heavier fractions. The trend of lower acid to aldehyde ratios in the recent soil (year 18) mirrors the input of fresh plant material, which constitutes relative higher aldhyde concentrations. Decreasing syringyl to vanillyl ratios (S/V) with increasing density (Figure 4b) also indicate advanced lignin degradation in heavier fractions, here because of preferential degradation of syringyl units. The divergence between the years can be explained by the input of the fresh plant material, which is abundant in syringyl units.

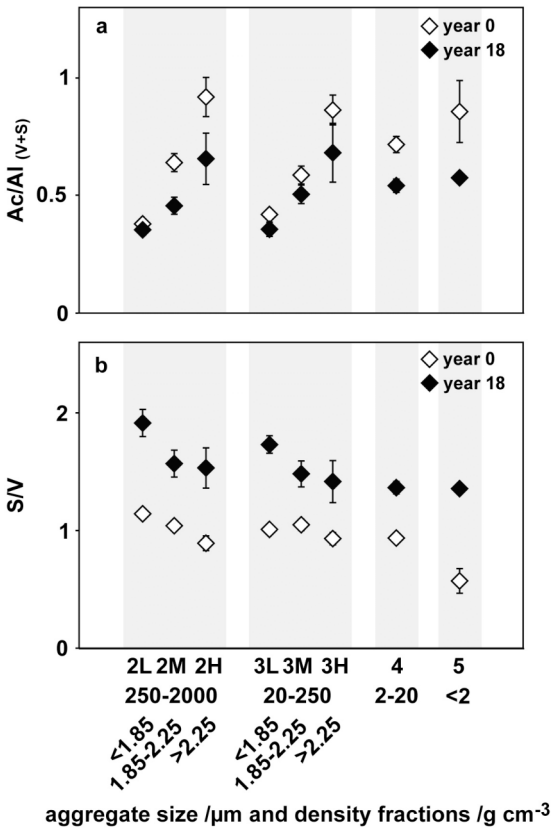

Figure 4 Indicators for lignin degradation in soil: **4a** Increasing acid to aldehyde ratios ($Ac/Al_{(V+S)}$) with increasing density indicate reduced aldehyde concentrations in heavier fractions due to advanced oxidation. **4b** Decreasing syringyl to vanillyl ratios (S/V) with increasing density also indicate advanced lignin degradation in heavier fractions.

Characterization of the fractions

Concentrations both of total SOC and lignin as well as of old, C_3-derived SOC and lignin decreased with fraction density (Table 1). Lignin carbon as determined with the CuO oxidation method represented about 8 mass-% of total SOC in the coarse light fraction (Table 1).

The fraction with the highest mass abundance was the coarse heavy fraction, which constituted about 60 % of the total soil mass, whereas particulate organic matter in the light fractions was very small in mass, contributing < 1 mass-% of total soil mass (Table 2).

Analyses of the mineralogy of the fractions by XRD Rietveld analysis revealed that light and medium fractions were similar in mineral composition, whereas the heavy fractions could clearly be differentiated. Heavy fractions contained least clay minerals (sum of illite, smectite, chlorite, kaolinite <10 mass-%) while holding ca 80 mass-% of quartz (Table 2). The differentiation of the heavy fractions was also supported by measurement of the specific surface area (SSA), which was significantly smaller in heavy fractions in comparison to light or fine textured fractions. The low specific surface area in heavy fractions results from the high quartz contents in combination with the relatively coarse texture (Table 2).

A further characterization of the coarse heavy fraction showed that this fraction consisted of about 52 mass-% aggregates and 39 mass-% mineral particles. The aggregates contained 80 % of the carbon stock in the 2H fractions, whereas the mineral particles contained only 3 % (Table 3).

Discussion

1. Coarse heavy fraction: low lignin concentration but large stock

In this context 'lignin concentration' refers to the content of lignin carbon (C_{VSC}) in a certain particle size or density fraction of the total soil. As we could show in Figure 2, the coarse heavy fraction (250 – 2000 μm, r > 2.25 g cm^{-3}) was the largest stock both for lignin carbon and for SOC at the beginning. The large mass of this fraction was the reason why, despite low concentrations (Table 1), the coarse heavy fraction constituted such a large stock for lignin and SOC. This finding leads to the question in what form lignin might have been stored in the coarse heavy fraction. Possible options include that lignin or lignin fragments might be stored (i) as occluded POM protected by aggregation (Golchin et al., 1994) or (ii) within organic matter layers on mineral surfaces (Baldock & Skjemstad, 2000; Kleber et al., 2007). The material of this fraction was dominated by individual sand particles (quartz, feldspars; Figure 5e) with only small amounts of clay minerals (Table 2). Despite the large proportion of mineral particles we found that the fraction was partly aggregated (macroaggregates, Figure 5e). Because ultrasonification was not applied, the waterstable aggregates that were recovered after sieving represented relatively stable aggregates in soil that might indeed have physically protected organic matter trapped within (Golchin et al., 1994; Six et al., 1998; Baldock & Skjemstad, 2000). We thus suggest that lignin was bound in aggregates and not adsorbed to mineral surfaces. We can support this suggestion by the low carbon concentrations of the mineral particles isolated from the coarse heavy fraction in comparison to the high concentrations in aggregates, which accounted for 80 mass-% of the total SOC in the coarse heavy fraction (Table 3). We therefore propose that SOC and lignin in the coarse heavy fractions are located in the macroaggregates, and not attached to quartz or feldspar particles.

2. Clay within aggregates might retain SOC and lignin

In order to explain how the C_3-derived lignin and SOC measured in the aggregates might have been stabilized over the course of 18 years, we calculated the mass fractions of lignin and SOC that could be explained by association with clay-sized minerals (Figure 6a, b). The association of carbon with clay minerals seemed to be more important in the heavier fractions (Figure 6) that at the same time accounted for the lowest concentration of clay minerals (Table 2). This result would actually support the importance of aggregates for SOC and lignin stocks in the heavy fraction because clay minerals in the heavy fraction should be bound in the aggregates due to the setup of the fractionation procedure without ultrasonic treatment (aim: preservation of waterstable aggregates). The correlation of high carbon enrichment in soils with low clay contents (Christensen & Sørensen, 1985) has been reviewed by Christensen (1996b) and could in analogy be applied to those physical soil fractions with low clay contents, i.e. carbon in the heavy fraction is associated with clay minerals that are part of soil aggregates.

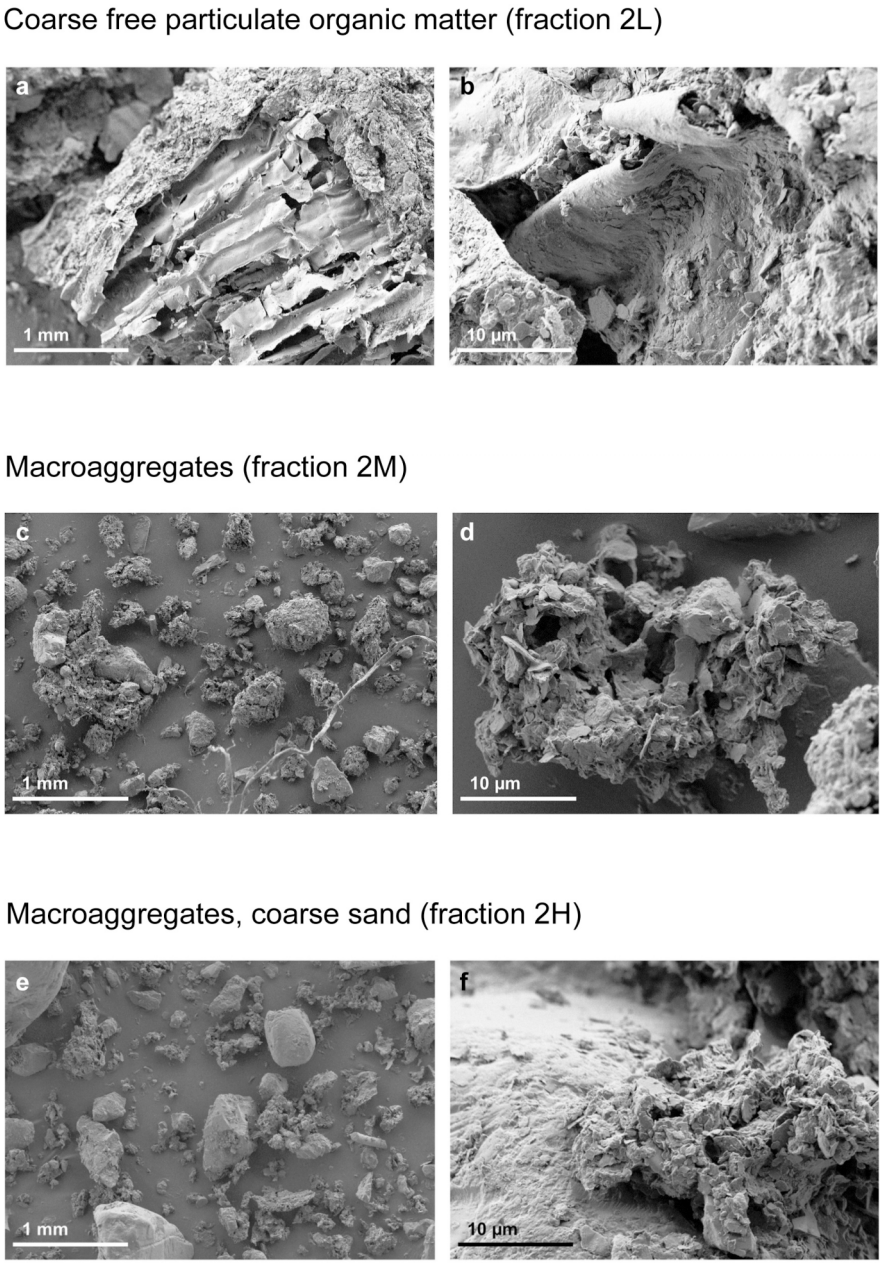

Figure 5 Scanning electron microscope (SEM) images for density fractions of the size fraction 250 – 2000 *µ*m taken with a secondary electron detector (SE; electron acceleration 2 keV). **5a, b** fraction density r < 1.85 g cm^{-3}, **5c, d** fraction density r 1.85 – 2.25 g cm^{-3}, **5e, f** fraction density r > 2.25 g cm^{-3}. Light fractions consisted of free particulate organic matter (fPOM), still recognizable as plant derived material, e.g. cell walls (**5a, b**), which are partly covered with mineral particles (**5b**). The medium density fraction consists mainly of aggregates (**5c, d**) while in the heavy fraction both aggregates and mineral particles occur.

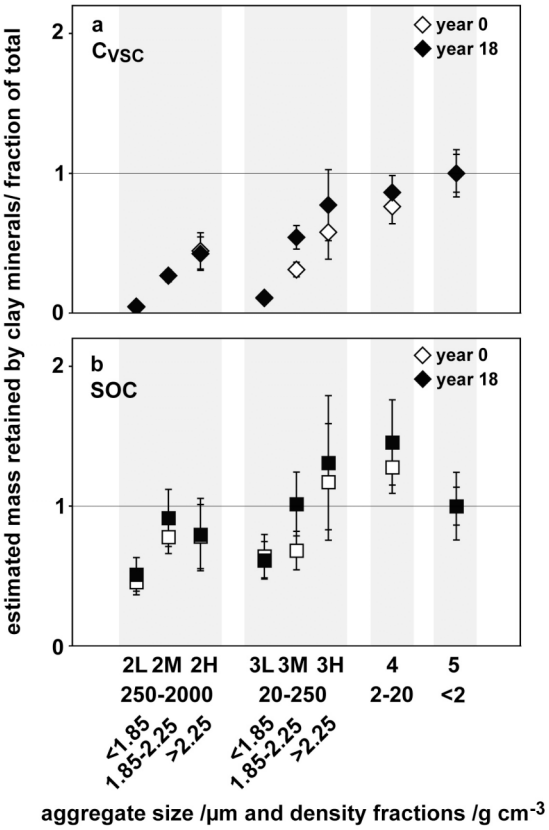

Figure 6 Estimated proportion of total mass of SOC and lignin that could be retained by clay minerals within each soil fraction. Values > 1 (line) suggest that adsorption to clay minerals might explain all carbon retention in that fraction, values < 1 suggest that clay minerals cannot explain all retained carbon, there must be other mechanisms.

3. Stocks and stability are not related

Stability here refers to the situation where no relative changes of the initial stocks of the labeled lignin can be measured in a fraction. A question one might ask is, if the heavy fraction might hold large stocks because the lignin is very stable in this fraction. The answer to this must clearly be no, because we found that more than 80 mass-% of the lignin in the heavy fractions was degraded within 18 years (Figure 3). Lignin in small size fractions (2-20 μm) appeared to be more stable, which would support the results of Heim and Schmidt 2007b, who presented evidence that lignin is preserved in the silt fraction. However, in the sandy soil of this study the heavy fractions were quantitatively more important than the fine particle size fractions and thus also preserved more lignin. With increasing density the fractions showed a trend of higher acid to aldehyde ratios (Figure 4a) and lower syringyl to vanillyl ratios (Figure 4b), which represent an advanced state of lignin degradation in the more mineral-associated fractions. We can suggest that lignin is altered most in the heavier fractions because these fractions already contain organic material that is in an advanced state of decomposition. Further decomposition of lignin could then be facilitated. Decreasing syringyl to vanillyl ratios with decreasing particle size are in accordance with Guggenberger et al. (1994) and also point to a more advanced state of lignin degradation in mineral-associated fractions and relatively less degradation in the lighter fraction.

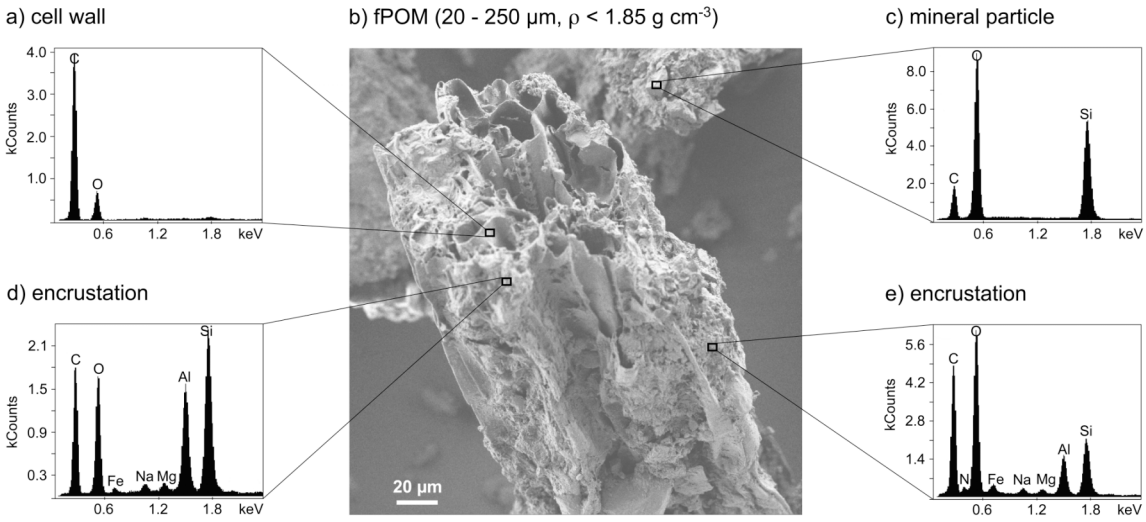

Figure 7 Chemical characterization of free particulate organic matter (size class 20 – 250 *µ*m, density ρ < 1.85 g cm-3) covered with a mineral-organic crust, as indicated by the occurrence of silicon, aluminum, iron and magnesium (**7d, e**). **7a, c, d and e** are graphs representing elements detected on the marked areas by EDX (Energy dispersive X-ray spectroscopy; electron acceleration 10 keV). Figure **7b** is a SEM image, taken with a secondary electron detector (electron acceleration 2 keV).

The suggested stability of lignin in the coarse light fraction (Figure 2b, 3) was somewhat unexpected because the plant material (particulate organic matter, Figure 5a, b) in this fraction is thought to turn over within few years (von Lützow et al., 2007; Marschner et al., 2008). We suggest three possible explanations for this finding:
(i) The stable lignin might be related to a recalcitrant subfraction of the lignin. The outer part of the macromolecule might be advanced in decomposition while the “core” might decompose more slowly because of lower accessibility for degrading enzymes.

(ii) The degradation of POM material might be hindered because of mineral encrustation (Figure 7). These crusts of mineral particles might act as a physical protection like an early state of aggregate formation (Baldock & Skjemstad, 2000). We found the aggregated POM because we chose a relatively high density cutoff (1.85 g cm^{-3}) for separation of POM in this sandy arable soil and thus separated POM that was associated with mineral particles (Figure 5a, b).

(iii) The stability of the lignin in the coarse light fraction could be an *apparent* stability. During the 18 years between the two samplings, aggregate turnover in the field might have caused a redistribution of lignin from the aggregates to light fractions. Thus the newly released inter-aggregate POM would have been protected also by spatial inaccessibility, also due to aggregation.

Conclusions

1. Distribution

Largest stocks of lignin and SOC in this sandy arable soil were in the coarse heavy fraction (250 - 2000 *µ*m, ρ > 2.25 g cm^{-3}), which was dominated by quartz and feldspar particles but also contained macro-aggregates. Most of the organic carbon within this fraction seemed to be stabilized by aggregation; sorption to individual quartz and feldspar particles was marginal. Smallest lignin and SOC stocks were found in the clay- and silt-sized fractions and in the light fractions (free particulate organic matter, 250 - 2000 *µ*m or 20 – 250 *µ*m, ρ <1.85 g cm^{-3}) because these fractions contributed only very small masses in the total soil.

2. Stock changes

Stock changes of C_3-labelled old lignin over 18 years were lowest in the silt-sized fraction and in the coarse light fraction. Stocks decreased markedly in all other fractions. We suggest that the particulate organic matter in the light fraction might have been preserved because of spatial inaccessibility on a macro-molecular or aggregate scale (encrustation with minerals), while an already advanced state of decomposition in the heavier fractions might have facilitated further lignin decomposition.

Acknowledgements

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Table 1 Concentrations of SOC and lignin in fractions. C_3-SOC and C_3-lignin are at least 18 years old and originate from the C_3-vegetation before labelling with maize (C_4) was started. Values are means of n = 2 analytical replicates with standard errors.

Fraction	Code	total SOC / mg g^{-1} fraction ± SE		C_3-SOC / mg g^{-1} fraction ± SE	total C_{VSC} / mg g^{-1} fraction ± SE		/ mg g^{-1} SOC ± SE [1]		C_3-C_{VSC} / mg g^{-1} fraction ± SE	/ mg g^{-1} SOC ± SE [1]	/ mg g^{-1} C_3-SOC ± SE [1]
		1988	2006	2006	1988	2006	1988	2006	2006	2006	2006
coarse sand size fraction 250 - 2000 µm											
< 1.85 g cm^{-3}	**2L**	157.5 ± 20.6	158.3 ± 12.8	15.8 ± 19.5	12.8 ± 0.3	22.6 ± 0.8	81.4 ± 10.8	143.0 ± 12.6	3.6 ± 1.0	23.0 ± 6.8	231.2 ± 292.6
1.85 - 2.25 g cm^{-3}	**2M**	91.1 ± 4.3	87.4 ± 6.2	45.9 ± 7.3	2.3 ± 0.0	3.6 ± 0.1	25.3 ± 1.3	41.5 ± 3.3	0.4 ± 0.1	4.4 ± 0.9	8.4 ± 2.1
> 2.25 g cm^{-3}	**2H**	17.2 ± 1.4	19.1 ± 1.7	13.6 ± 1.8	0.3 ± 0.0	0.4 ± 0.0	15.4 ± 1.4	22.9 ± 2.3	0.0 ± 0.0	1.8 ± 0.7	2.5 ± 1.0
fine sand size fraction 20 - 250 µm											
< 1.85 g cm^{-3}	**3L**	136.1 ± 23.8	160.7 ± 0.9	83.3 ± 14.4	6.8 ± 0.1	11.2 ± 0.2	49.7 ± 8.7	69.5 ± 1.2	2.3 ± 0.5	14.2 ± 3.3	27.5 ± 8.0
1.85 - 2.25 g cm^{-3}	**3M**	122.8 ± 14.0	92.9 ± 4.7	65.5 ± 5.3	2.3 ± 0.0	2.1 ± 0.1	19.0 ± 2.2	22.8 ± 1.3	0.4 ± 0.0	4.3 ± 0.5	6.1 ± 0.8
> 2.25 g cm^{-3}	**3H**	14.1 ± 1.8	14.2 ± 0.9	10.9 ± 0.9	0.2 ± 0.0	0.3 ± 0.0	17.7 ± 2.3	20.8 ± 1.7	0.0 ± 0.0	2.9 ± 1.2	3.8 ± 1.6
Silt size fraction 2 - 20 µm	**4**	55.5 ± 1.1	54.8 ± 3.4	44.3 ± 3.6	0.8 ± 0.0	1.1 ± 0.0	14.6 ± 0.4	20.6 ± 1.3	0.7 ± 0.1	12.3 ± 1.2	15.3 ± 1.7
Clay size fraction 0.45 - 2 µm	**5**	95.3 ± 0.6	107.2 ± 15.6	88.3 ± 15.9	0.8 ± 0.1	1.3 ± 0.0	8.7 ± 0.7	12.3 ± 1.8	0.5 ± 0.1	4.3 ± 0.8	5.2 ± 1.1

[1] includes error propagation

Table 2 Mass distribution, specific surface area and mineralogy of the fractions. For mass distribution: standard error of three replicate wet sievings or two replicate density fractionations (analytical replicates of one homogenized soil sample from the archive). For mineralogy: standard error from Rietveld analysis (one analytical replicate), soil samples are from year 18, except 3L (year 0) because too little amount was available from the year 18 sample. For specific surface area: standard error of two analytical replicates, sample amounts were in some cases (n.d.) too small or already milled which prevented the use for surface analysis.

Fraction	Code	Mass distribution		Mineralogy (XRD)			Specific surface area (N_2-BET)	
				quartz	feldspars, muscovite	clay minerals[1]		
		/ mass-% of bulk soil ± SE		/ % of fraction ± SE	/ % of fraction ± SE	/ % of fraction ± SE	/ m^{-2} g^{-1} fraction ± SE	
		1988	2006	2006	2006	2006	1988	2006
coarse sand size fraction 250 - 2000 µm								
< 1.85 g cm^{-3}	**2L**	0.3 ± 0.0	0.8 ± 0.1	17.3 ± 1.4	16.6 ± 3.2	30.5 ± 5.0	n.d.	4.8 ± 0.9
1.85 - 2.25 g cm^{-3}	**2M**	2.8 ± 0.1	2.2 ± 0.1	26.5 ± 1.1	14.8 ± 1.9	30.2 ± 4.2	3.8 ± 0.5	4.9 ± 0.5
> 2.25 g cm^{-3}	**2H**	55.9 ± 2.6	64.3 ± 2.0	81.2 ± 2.0	12.6 ± 1.3	5.7 ± 2.0	0.7 ± 0.0	0.9 ± 0.0
fine sand size fraction 20 - 250 µm								
< 1.85 g cm^{-3}	**3L**	0.2 ± 0.0	0.1 ± 0.0	14.7 ± 1.2	14.3 ± 2.8	37.1 ± 5.2	n.d.	17.8 ± 0.5
1.85 - 2.25 g cm^{-3}	**3M**	3.9 ± 0.3	2.2 ± 0.2	23.8 ± 0.8	17.3 ± 1.7	35.5 ± 5.1	3.2 ± 0.0	3.7 ± 0.1
> 2.25 g cm^{-3}	**3H**	30.8 ± 2.3	26.6 ± 1.9	77.4 ± 1.9	15.1 ± 1.7	7.0 ± 4.3	0.7 ± 0.0	1.0 ± 0.0
silt size fraction 2 - 20 µm	**4**	1.6 ± 0.1	1.4 ± 0.0	38.9 ± 1.1	18.1 ± 1.6	30.1 ± 4.7	2.8 ± 0.1	3.4 ± 0.0
clay size fraction 0.45 - 2 µm	**5**	0.1 ± 0.0	0.1 ± 0.0	19.8 ± 0.8	17.8 ± 2.0	40.4 ± 4.5	2.5 ± n.d.	8.1 ± n.d.

n.d. not determined

[1] Σ illite, smectite, chlorite, kaolinite

Table 3 Density subfractions of coarse heavy fraction (2H, 250 - 2000 µm, $\rho > 2.25$ g cm^{-3}).

Subfraction	Mass distribution / mass-% in fraction 2H	SOC concentration / mg g^{-1} subfraction	/ mg g^{-1} fraction 2H	/ mass-% in fraction 2H
aggregates, iPOM (ρ = 2.25 - 2.65 g cm^{-3})	52	29.7	15.5	80
mineral particles ($\rho > 2.65$ g cm^{-3})	39	1.5	0.6	3
Released clay + silt (ρ = 2.25 - 2.65 g cm^{-3})[1]	9	36.0	3.2	17

[1] recovered from density solution (size 0.45 - 32 µm)

Part C Appendix

Table 1 Data Review on lignin concentrations in mineral soil of different land uses. Lignin measured as VSC-lignin after CuO oxidation. Standard errors in parentheses where available.

Reference	Lignin (VSC)		SOC	Land use	Sampling depth	Location
	/mg g^{-1} SOC	/mg kg^{-1} soil	/g kg^{-1} soil		/cm(or horizon)	
Arable soils						
Guggenberger et al. (1994)	10.9			arable rotation since 1905	A	Bavaria, Germany
Jolivet et al. (2001)	8.1			Maize 4 y	0-25	Gascogny, France
	9.1			Maize 22 y	0-25	Gascogny, France
Lobe et al. (2002)	9.5 (0.8)	42 (10)	4.4	arable rotation 90-98 y	0-20	Free State Province, South Africa (subtropic)
Kiem and Kögel-Knabner (2003)				*fertilized*		
	21.7	150	6.8	arable rotation since 1937	0-20	Thyrow, Germany
	25.4	260	10	arable rotation since 1967	0-20	Groß Kreutz, Germany
	13.2	120	8.8	arable rotation since 1923	0-20	Skierniewice, Poland
	15.9	190	12	arable rotation since 1983	0-20	Puch, Germany
	16.8	810	48	arable rotation since 1966	0-20	Lauterbach, Germany
	19.1	460	24	arable rotation since 1902	0-20	Bad Lauchstädt A, Germany
	28.9	1190	41	arable rotation since 1983, fertilized with 200 t ha^{-1} y^{-1} farmyard manure	0-20	Bad Lauchstädt B, Germany
	26	760	29	bare fallow since 1958, fertilized with 70 t ha^{-1} y^{-1} farmyard manure	0-20	Prague, Czech Republic
				depleted		
	4.9	20	3.2	arable rotation since 1937	0-20	Thyrow, Germany
	13	50	4.1	arable rotation since 1967	0-20	Groß Kreutz, Germany
	4.6	20	4.4	arable rotation since 1923	0-20	Skierniewice, Poland
	6.5	50	7	bare fallow since 1983	0-20	Puch, Germany
	8	24	30	arable rotation since 1966	0-20	Lauterbach, Germany
	5.4	90	16	arable rotation since 1902	0-20	Bad Lauchstädt A, Germany
	1.7	30	15	bare fallow since 1902	0-20	Bad Lauchstädt A, Germany
	9.8	190	20	bare fallow since 1983	0-20	Bad Lauchstädt B, Germany
	5.1	80	14	bare fallow since 1958	0-20	Prague, Czech Republic
Leifeld and Kögel-Knabner (2005)	29.7 (4.6)			arable rotation > 20y	0-3	Scheyern, Bavaria, Germany
	32.3 (2.2)			arable rotation > 20y	0-3	Scheyern, Bavaria, Germany
	31.6 (4.5)			arable rotation > 20y	0-3	Scheyern, Bavaria, Germany
Dignac et al. (2005)	17.2			Wheat 9 y	0-25	"Les Closeaux" Grignon, France
	19			Maize 9 y	0-25	"Les Closeaux" Grignon, France
Heim and Schmidt (2007a)	26 (1.2)	303 (11)	11.6	Wheat 23 y	0-30	Boigneville, Essonne, France
	31 (2.0)	299 (20)	9.8	Maize 23 y	0-30	Boigneville, Essonne, France
Heim and Schmidt (2007b)	17.7	325	11.2	Wheat 33 y	0-30	Rotthalmünster, Bavaria, Germany
	23.8	442	11.3	Maize 23 y	0-30	Rotthalmünster, Bavaria, Germany
Bierke et al. (2008)				*paddy soil, rice straw removed*		
	48.1 (0.2)			1 y	0-20	Nanjing, China (subtropic)
	55.9 (0.2; min) - 61.3 (0.2; max)			2 y	0-20	Los Baños, Philippines (tropic)
	26.7 (2.0)			15 y	0-20	Changsa, China (subtropic)
				paddy soil, rice straw incorporated		
	48.7 (0.2)			1 y	0-20	Nanjing, China (subtropic)
	55.2 (0.2; min) - 63.8 (0.2; max)			2 y	0-20	Los Baños, Philippines (tropic)
	33.4 (3.0)			15 y	0-20	Changsa, China (subtropic)
Hofmann et al. (2009 a)	24.7 (1.6)	587 (33)	23.8 (0.8)	Maize 18 y, Askov soil, small input of crop residues	0-20	Askov, Denmark
(this thesis)	34.6 (1.4)	959 (40)	27.8 (0.2)	Maize 18 y, Askov soil, high input of crop residues	0-20	Askov, Denmark
	35.2 (1.7)	368 (3)	10.4 (0.5)	Maize 18 y, Lundgaard soil, small input of crop residues	0-20	Askov, Denmark
	44.9 (1.0)	645 (11)	14.4 (0.2)	Maize 18 y, Lundgaard soil, high input of crop residues	0-20	Askov, Denmark
Hofmann et al. (2009 b)	19.8	164	8.3	Maize 36 y, fertilized	0-25/ 0-35(45)	Cadriano, Bologna, Italy
(this thesis)	15.8	114	7.2	Maize 36 y, non-fertilized	0-25/ 0-35(45)	Cadriano, Bologna, Italy

Table 1 (continued)

Grassland soils						
Guggenberger et al. (1994)	19			permanent grassland on previous arable site, established in 1956	A	Bavaria, Germany
Sanger et al. (1997)	139 (2.4)			pasture	0-15	Kildare County, Ireland
Amelung and Zech (1999)						*climate sequence, North America:*
	19.6 (min) - 21.5 (max)			native grassland	0-10	cryic
	16.7 (min) - 26.4 (max)			native grassland	0-10	frigid
	11.5 (min) - 24.1 (max)			native grassland	0-10	mesic
	16.6 (min) - 23.8 (max)			native grassland	0-10	thermic
	9.1 (min) - 17.2 (max)			native grassland	0-10	hyperthermic
Lobe et al. (2002)	10.4 (0.9)	132 (29)	12.7	native grassland	0-20	Free State Province, South Africa
Leifeld and Kögel-Knabner (2005)	21.7 (3.1)			permanent grassland	0-3	Scheyern, Bavaria, Germany
Heim and Schmidt (2007a)	32	789 (34)	24.2	Pasture	0-10	Hohenheim, Baden-Württemberg, Germany
	49	787 (33)	16.2	Miscanthus	0-10	Hohenheim, Baden-Württemberg, Germany
	31	806 (27)	26	Ryegrass control	0-10	Eschikon, Zurich, Switzerland
	34	904 (55)	26.6	Ryegrass labelled	0-10	Eschikon, Zurich, Switzerland
	25	636 (30)	25.4	Clover control	0-10	Eschikon, Zurich, Switzerland
	30	663 (33)	22.1	Clover labelled	0-10	Eschikon, Zurich, Switzerland
Forest soils						
Kögel (1986)	12.8			Mixed deciduous forest	Ah	Bavaria, Germany
	16			Mixed deciduous forest	Ah	Bavaria, Germany
	10.2			Spruce forest	Aeh	Bavaria, Germany
Guggenberger et al. (1994)	22.6			Spruce forest	A	Bavaria, Germany
	12.0			Mixed deciduous forest	A	Bavaria, Germany
Sanger et al. (1997)	351 (39)			Ash forest	0-10	Kildare County, Ireland
	158 (17)			Spruce forest	0-5	Kildare County, Ireland
Jolivet et al. (2001)	8.9			Pine forest	0-25	Gascogny, France
Schmidt and Kögel-Knabner (2002)	8.5			Mixed deciduous forest	0-14	Siggen, Schleswig-Holstein, Germany
Sollins et al. (2006)				Mixed coniferous forest, six density fractions (no bulk soil value)	A	Oregon, USA
	7.8			< 1.65 g cm $^{-3}$		
	31.0			> 2.55 g cm $^{-3}$		

Kyoto Protocol, Article 3.4

Kyoto Protocol to the United Nations Framework Convention on Climate Change (UNFCCC)

Extract from Article 3.4:

"[…] The Conference of the Parties serving as the meeting of the Parties to this Protocol shall, at its first session or as soon as practicable thereafter, decide upon modalities, rules and guidelines as to how, and which, ***additional human-induced activities related to changes in greenhouse gas emissions by sources and removals by sinks in the agricultural soils and the land-use change and forestry categories shall be added to, or subtracted from, the assigned amounts*** for Parties included in Annex I, taking into account uncertainties, transparency in reporting, verifiability, the methodological work of the Intergovernmental Panel on Climate Change, the advice provided by the Subsidiary Body for Scientific and Technological Advice in accordance with Article 5 and the decisions of the Conference of the Parties. Such a decision shall apply in the second and subsequent commitment periods. […]"

Data supplement to manuscript I

Table 2 Soil organic carbon concentrations in Askov and Lundgaard soil samples.

Askov soil

Treatment	Year	total SOC /mg g^{-1} soil		C_4-SOC /mg g^{-1} soil		C_3-SOC /mg g^{-1} soil	
		Mean	SE [a]	Mean	SE [a]	Mean	SE [a]
control	0	25.6	0.5			25.6	0.5
low input	3	26.1	0.1	0.9	0.3	25.1	0.3
	6	24.5	0.1	1.8	0.1	22.6	0.1
	10	no sample available		no sample available		no sample available	
	15	25.4	0.1	3.7	0.2	21.7	0.2
	18	23.8	0.8	3.9	0.2	19.9	0.2
high input	3	27.6	0.2	2.9	0.2	24.7	0.2
	6	27.5	0.7	3.8	0.2	23.7	0.2
	10	27.0	0.2	4.4	0.3	22.6	0.3
	15	30.0	0.2	8.1	0.5	22.0	0.5
	18	27.8	0.2	8.6	0.5	19.2	0.5

[a] The standard error is the analytical error of two lab replicates of one homogenized archived soil sample per treatment and sampling date.

Lundgaard soil

Treatment	Year	total SOC /mg g^{-1} soil		C_4-SOC /mg g^{-1} soil		C_3-SOC /mg g^{-1} soil	
		Mean	SE [a]	Mean	SE [a]	Mean	SE [a]
control	0	11.0	0.3			11.0	0.3
low input	3	10.7	0.4	0.0	0.3	10.7	0.3
	6	11.8	0.2	0.2	0.4	11.7	0.4
	10	no sample available		no sample available		no sample available	
	15	10.9	0.5	1.5	0.4	9.3	0.4
	18	10.4	0.5	1.8	0.4	8.6	0.4
high input	3	11.6	0.5	0.5	0.4	11.2	0.4
	6	12.2	0.4	2.7	0.4	9.5	0.4
	10	12.5	0.2	2.5	0.4	10.0	0.4
	15	14.7	0.5	5.0	0.6	9.7	0.6
	18	14.4	0.2	5.2	0.6	9.2	0.6

[a] The standard error is the analytical error of two lab replicates of one homogenized archived soil sample per treatment and sampling date.

Table 3 VSC-lignin concentrations in Askov and Lundgaard soil samples.

Askov soil

Treatment	Year	total VSC / mg g^{-1} soil		C_4-VSC / mg g^{-1} soil		C_3-VSC / mg g^{-1} soil	
		Mean	SE [a]	Mean	SE [a]	Mean	SE [a]
control	**0**	0.510	0.025	0.000	0.000	0.510	0.025
low input	**3**	0.539	0.042	0.083	0.009	0.456	0.036
	6	0.454	0.031	0.119	0.017	0.334	0.015
	10	no sample available		no sample available		no sample available	
	15	0.519	0.026	0.235	0.018	0.284	0.009
	18	0.587	0.033	0.227	0.051	0.360	0.019
high input	**3**	0.663	0.026	0.173	0.019	0.491	0.033
	6	0.725	0.030	0.349	0.013	0.376	0.020
	10	0.666	0.027	0.347	0.015	0.318	0.018
	15	0.855	0.045	0.558	0.048	0.297	0.035
	18	0.959	0.040	0.527	0.034	0.433	0.036

[a] The standard error is the analytical error of six lab replicates (years 0 and 3) or three lab replicates (years 6, 10, 15, 18) of one homogenized archived soil sample per treatment and sampling date.

Lundgaard soil

Treatment	Year	total VSC / mg g^{-1} soil		C_4-VSC / mg g^{-1} soil		C_3-VSC / mg g^{-1} soil	
		Mean	SE [a]	Mean	SE [a]	Mean	SE [a]
control	**0**	0.267	0.010	0.000	0.000	0.267	0.010
low input	**3**	0.255	0.013	0.028	0.003	0.228	0.011
	6	0.249	0.009	0.058	0.008	0.190	0.013
	10	no sample available		no sample available		no sample available	
	15	0.341	0.013	0.179	0.004	0.162	0.015
	18	0.368	0.003	0.201	0.003	0.168	0.005
high input	**3**	0.315	0.008	0.075	0.008	0.240	0.010
	6	0.424	0.008	0.209	0.007	0.215	0.013
	10	0.403	0.037	0.208	0.014	0.195	0.023
	15	0.561	0.016	0.357	0.025	0.204	0.015
	18	0.645	0.011	0.384	0.022	0.262	0.032

[a] The standard error is the analytical error of six lab replicates (years 0 and 3) or three lab replicates (years 6, 10, 15, 18) of one homogenized archived soil sample per treatment and sampling date.

Table 4 VSC-lignin concentrations in SOC of Askov and Lundgaard soil samples.

Askov soil

Treatment	Year	total VSC / mg g^{-1} SOC		C_4-VSC / mg g^{-1} SOC		C_3-VSC / mg g^{-1} SOC	
		Mean	SE [a]	Mean	SE [a]	Mean	SE [a]
control	0	19.9	1.0	0.0	0.0	19.9	1.0
low input	3	20.7	1.6	3.2	0.3	17.5	1.4
	6	18.5	1.3	4.9	0.7	13.7	0.6
	10	no sample available		no sample available		no sample available	
	15	20.4	1.0	9.2	0.7	11.2	0.4
	18	24.7	1.6	9.5	2.2	15.1	0.9
high input	3	24.0	1.0	6.2	0.7	17.8	1.2
	6	26.4	1.3	12.7	0.6	13.7	0.8
	10	24.7	1.0	12.9	0.6	11.8	0.7
	15	28.5	1.5	18.6	1.6	9.9	1.2
	18	34.6	1.4	19.0	1.2	15.6	1.3

[a] The standard error includes error propagation.

Lundgaard soil

Treatment	Year	total VSC / mg g^{-1} SOC		C_4-VSC / mg g^{-1} SOC		C_3-VSC / mg g^{-1} SOC	
		Mean	SE [a]	Mean	SE [a]	Mean	SE [a]
control	0	24.2	1.1	0.0	0.0	24.2	1.1
low input	3	23.9	1.4	2.6	0.3	21.3	1.2
	6	21.0	0.9	4.9	0.7	16.1	1.1
	10	no sample available		no sample available		no sample available	
	15	31.4	1.8	16.5	0.8	15.0	1.5
	18	35.2	1.7	19.2	0.9	16.0	0.9
high input	3	27.1	1.4	6.4	0.8	20.7	1.3
	6	34.8	1.3	17.2	0.8	17.6	1.2
	10	32.1	2.9	16.6	1.2	15.5	1.8
	15	38.2	1.6	24.3	1.9	13.9	1.1
	18	44.9	1.0	26.7	1.6	18.2	2.3

[a] The standard error includes error propagation.

Table 5 Lignin carbon (C_{VSC}) concentrations in Askov and Lundgaard soil samples.

Askov soil

Treatment	Year	C_{VSC} total /mg g^{-1} soil		$C_{C4\text{-}VSC}$ /mg g^{-1} soil		$C_{C3\text{-}VSC}$ /mg g^{-1} soil	
		Mean	SE [a]	Mean	SE [a]	Mean	SE [a]
control	0	0.309	0.014	0.000	0.000	0.309	0.014
low input	3	0.327	0.025	0.051	0.005	0.277	0.022
	6	0.277	0.019	0.074	0.010	0.203	0.009
	10	no sample available		no sample available		no sample available	
	15	0.317	0.015	0.144	0.011	0.173	0.006
	18	0.357	0.019	0.139	0.031	0.208	0.011
high input	3	0.404	0.016	0.106	0.012	0.298	0.020
	6	0.444	0.018	0.214	0.008	0.229	0.013
	10	0.408	0.016	0.214	0.009	0.194	0.011
	15	0.523	0.028	0.342	0.029	0.181	0.022
	18	0.584	0.024	0.321	0.021	0.243	0.009

[a] The standard error is the analytical error of six lab replicates (years 0 and 3) or three lab replicates (years 6, 10, 15, 18) of one homogenized archived soil sample per treatment and sampling date.

Lundgaard soil

Treatment	Year	C_{VSC} total /mg g^{-1} soil		$C_{C4\text{-}VSC}$ /mg g^{-1} soil		$C_{C3\text{-}VSC}$ /mg g^{-1} soil	
		Mean	SE [a]	Mean	SE [a]	Mean	SE [a]
control	0	0.161	0.006	0.000	0.000	0.161	0.006
low input	3	0.155	0.007	0.017	0.002	0.138	0.006
	6	0.151	0.006	0.036	0.005	0.116	0.008
	10	no sample available		no sample available		no sample available	
	15	0.207	0.008	0.109	0.002	0.098	0.009
	18	0.224	0.002	0.122	0.002	0.102	0.003
high input	3	0.191	0.005	0.046	0.005	0.146	0.006
	6	0.258	0.005	0.128	0.004	0.130	0.008
	10	0.246	0.022	0.127	0.009	0.119	0.014
	15	0.342	0.010	0.218	0.016	0.124	0.009
	18	0.393	0.006	0.233	0.014	0.140	0.002

[a] The standard error is the analytical error of six lab replicates (years 0 and 3) or three lab replicates (years 6, 10, 15, 18) of one homogenized archived soil sample per treatment and sampling date.

Table 6 Lignin carbon (C_{VSC}) concentrations in SOC of Askov and Lundgaard soil samples.

Askov soil

Treatment	Year	C_{VSC} total /mg g^{-1} SOC		$C_{C4\text{-}VSC}$ /mg g^{-1} SOC		$C_{C3\text{-}VSC}$ /mg g^{-1} SOC	
		Mean	SE [a]	Mean	SE [a]	Mean	SE [a]
control	0	12.1	0.6	0.0	0.0	12.1	0.6
low input	3	12.6	1.0	1.9	0.2	10.6	0.8
	6	11.3	0.8	3.0	0.4	8.3	0.4
	10	no sample available		no sample available		no sample available	
	15	12.5	0.6	5.7	0.4	6.8	0.2
	18	15.0	0.9	5.9	1.3	8.8	0.5
high input	3	14.6	0.6	3.8	0.4	10.8	0.7
	6	16.1	0.8	7.8	0.3	8.3	0.5
	10	15.1	0.6	7.9	0.3	7.2	0.4
	15	17.4	0.9	11.4	1.0	6.0	0.7
	18	21.0	0.9	11.6	0.8	8.8	0.3

[a] The standard error includes error propagation.

Lundgaard soil

Treatment	Year	C_{VSC} total /mg g^{-1} SOC		$C_{C4\text{-}VSC}$ /mg g^{-1} SOC		$C_{C3\text{-}VSC}$ /mg g^{-1} SOC	
		Mean	SE [a]	Mean	SE [a]	Mean	SE [a]
control	0	14.7	0.7	0.0	0.0	14.7	0.7
low input	3	14.5	0.9	1.6	0.2	12.9	0.7
	6	12.8	0.5	3.0	0.4	9.8	0.7
	10	no sample available		no sample available		no sample available	
	15	19.1	1.1	10.0	0.5	9.1	0.9
	18	21.4	1.0	11.7	0.6	9.8	0.5
high input	3	16.4	0.8	3.9	0.5	12.5	0.8
	6	21.2	0.8	10.5	0.5	10.7	0.8
	10	19.6	1.8	10.1	0.7	9.5	1.1
	15	23.3	1.0	14.8	1.2	8.5	0.7
	18	27.3	0.6	16.2	1.0	9.8	0.2

[a] The standard error includes error propagation.

Table 7 Lignin monomer concentrations in soil samples.

Askov soil

Lignin monomers (total)	Concentration/ mg g^{-1} soil																					
	control		low input										high input									
	0		3		6		10		15		18		3		6		10		15		18	
	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]
Vanillin (Vl)	0.108	0.002	0.109	0.004	0.097	0.008	n.a.	n.a.	0.104	0.005	0.105	0.002	0.133	0.002	0.144	0.008	0.127	0.005	0.152	0.009	0.153	0.004
Vanillic acid (Vd)	0.060	0.010	0.058	0.009	0.036	0.004	n.a.	n.a.	0.042	0.007	0.068	0.001	0.064	0.010	0.047	0.005	0.040	0.004	0.047	0.007	0.088	0.005
Acetovanillone (Vn)	0.044	0.003	0.043	0.004	0.033	0.004	n.a.	n.a.	0.036	0.003	0.043	0.000	0.049	0.002	0.046	0.004	0.040	0.002	0.048	0.003	0.058	0.001
Syringaldehyde (Sl)	0.092	0.001	0.101	0.003	0.098	0.008	n.a.	n.a.	0.123	0.006	0.121	0.001	0.128	0.003	0.159	0.008	0.151	0.009	0.199	0.012	0.201	0.008
Syringic acid (Sd)	0.068	0.009	0.067	0.012	0.043	0.008	n.a.	n.a.	0.052	0.008	0.059	0.018	0.077	0.010	0.074	0.008	0.061	0.006	0.094	0.012	0.123	0.016
Acetosyringone (Sn)	0.060	0.005	0.064	0.007	0.047	0.003	n.a.	n.a.	0.057	0.004	0.090	0.002	0.081	0.007	0.076	0.004	0.072	0.003	0.096	0.008	0.146	0.008
p-Coumaric acid (pCd)	0.031	0.002	0.044	0.004	0.044	0.003	n.a.	n.a.	0.053	0.005	0.068	0.003	0.062	0.005	0.096	0.004	0.094	0.006	0.118	0.007	0.136	0.007
Ferulic acid (Fd)	0.048	0.005	0.053	0.002	0.056	0.002	n.a.	n.a.	0.053	0.001	0.032	0.014	0.070	0.007	0.083	0.006	0.079	0.005	0.101	0.003	0.055	0.015
$Ac/Al_{(V)}$	0.6	0.1	0.5	0.1	0.4	0.1	n.a.	n.a.	0.4	0.1	0.6	0.0	0.5	0.1	0.3	0.0	0.3	0.0	0.3	0.0	0.6	0.0
$Ac/Al_{(S)}$	0.7	0.1	0.7	0.1	0.4	0.1	n.a.	n.a.	0.4	0.1	0.5	0.1	0.6	0.1	0.5	0.1	0.4	0.0	0.5	0.1	0.6	0.1
$Ac/Al_{(V+S)}$	0.6	0.1	0.6	0.1	0.4	0.1	n.a.	n.a.	0.4	0.0	0.6	0.1	0.5	0.1	0.4	0.0	0.4	0.0	0.4	0.0	0.6	0.0
S/V	1.0	0.1	1.1	0.1	1.1	0.1	n.a.	n.a.	1.3	0.1	1.3	0.1	1.2	0.1	1.3	0.1	1.4	0.1	1.6	0.1	1.6	0.1
C/V	0.4	0.0	0.5	0.0	0.6	0.0	n.a.	n.a.	0.6	0.0	0.5	0.1	0.5	0.0	0.8	0.0	0.8	0.0	0.9	0.1	0.6	0.1

[a] The standard error is the analytical error of six lab replicates (years 0 and 3) or three lab replicates (years 6, 10, 15, 18) of one homogenized archived soil sample per treatment and sampling date.

n.a. sample not available

Lundgaard soil

Lignin monomers (total)	Concentration/ mg g^{-1} soil																					
	control		low input										high input									
	0		3		6		10		15		18		3		6		10		15		18	
	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]
Vanillin (Vl)	0.060	0.001	0.056	0.002	0.054	0.003	n.a.	n.a.	0.064	0.002	0.063	0.002	0.067	0.001	0.080	0.003	0.075	0.006	0.096	0.002	0.106	0.002
Vanillic acid (Vd)	0.028	0.003	0.026	0.004	0.021	0.003	n.a.	n.a.	0.025	0.003	0.030	0.002	0.029	0.002	0.027	0.004	0.023	0.003	0.031	0.001	0.046	0.002
Acetovanillone (Vn)	0.020	0.001	0.019	0.001	0.017	0.001	n.a.	n.a.	0.020	0.001	0.021	0.001	0.023	0.001	0.024	0.002	0.022	0.002	0.030	0.001	0.035	0.000
Syringaldehyde (Sl)	0.055	0.001	0.053	0.002	0.056	0.002	n.a.	n.a.	0.082	0.001	0.091	0.003	0.067	0.001	0.094	0.002	0.094	0.008	0.136	0.003	0.152	0.007
Syringic acid (Sd)	0.036	0.002	0.034	0.003	0.029	0.001	n.a.	n.a.	0.044	0.003	0.045	0.004	0.040	0.002	0.050	0.003	0.044	0.004	0.066	0.003	0.083	0.006
Acetosyringone (Sn)	0.033	0.003	0.032	0.003	0.028	0.001	n.a.	n.a.	0.040	0.002	0.051	0.000	0.040	0.002	0.052	0.001	0.049	0.005	0.072	0.002	0.087	0.005
p-Coumaric acid (pCd)	0.012	0.001	0.016	0.001	0.020	0.002	n.a.	n.a.	0.038	0.003	0.046	0.003	0.025	0.001	0.051	0.005	0.053	0.009	0.073	0.007	0.095	0.007
Ferulic acid (Fd)	0.021	0.002	0.020	0.002	0.023	0.002	n.a.	n.a.	0.028	0.002	0.023	0.004	0.026	0.002	0.045	0.005	0.043	0.004	0.058	0.003	0.040	0.001
$Ac/Al_{(V)}$	0.5	0.0	0.5	0.1	0.4	0.1	n.a.	n.a.	0.4	0.1	0.5	0.0	0.4	0.0	0.3	0.0	0.3	0.0	0.3	0.0	0.4	0.0
$Ac/Al_{(S)}$	0.7	0.0	0.6	0.1	0.5	0.0	n.a.	n.a.	0.5	0.0	0.5	0.0	0.6	0.0	0.5	0.0	0.5	0.1	0.5	0.0	0.5	0.0
$Ac/Al_{(V+S)}$	0.6	0.0	0.6	0.0	0.4	0.0	n.a.	n.a.	0.5	0.0	0.5	0.0	0.5	0.0	0.4	0.0	0.4	0.0	0.4	0.0	0.5	0.0
S/V	1.1	0.0	1.2	0.1	1.2	0.1	n.a.	n.a.	1.5	0.1	1.6	0.1	1.2	0.0	1.5	0.1	1.6	0.1	1.7	0.0	1.7	0.1
C/V	0.3	0.0	0.4	0.0	0.5	0.0	n.a.	n.a.	0.6	0.0	0.6	0.0	0.4	0.0	0.7	0.1	0.8	0.1	0.8	0.0	0.7	0.0

[a] The standard error is the analytical error of six lab replicates (years 0 and 3) or three lab replicates (years 6, 10, 15, 18) of one homogenized archived soil sample per treatment and sampling date.

n.a. sample not available

Table 8 C_4-derived lignin monomers in soil samples.

Askov soil

Lignin monomers (C_4-derived)	Concentration/ mg g^{-1} soil																					
	control		low input										high input									
	0		3		6		10		15		18		3		6		10		15		18	
	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]
Vanillin (Vl)	0.000	0.000	0.009	0.001	0.015	0.000	n.a.	n.a.	0.033	0.004	0.027	0.003	0.023	0.002	0.053	0.008	0.047	0.007	0.079	0.013	0.071	0.008
Vanillic acid (Vd)	0.000	0.000	0.004	0.000	0.005	0.003	n.a.	n.a.	0.012	0.002	0.008	0.003	0.006	0.001	0.014	0.002	0.013	0.000	0.023	0.005	0.019	0.004
Acetovanillone (Vn)	0.000	0.000	0.003	0.001	0.004	0.001	n.a.	n.a.	0.010	0.002	0.009	0.002	0.007	0.001	0.014	0.002	0.013	0.001	0.024	0.004	0.019	0.004
Syringaldehyde (Sl)	0.000	0.000	0.028	0.008	0.028	0.007	n.a.	n.a.	0.063	0.006	0.048	0.025	0.036	0.008	0.088	0.006	0.092	0.005	0.148	0.014	0.125	0.047
Syringic acid (Sd)	0.000	0.000	0.006	0.001	0.007	0.003	n.a.	n.a.	0.020	0.003	0.021	0.007	0.015	0.001	0.029	0.003	0.026	0.003	0.049	0.005	0.069	0.015
Acetosyringone (Sn)	0.000	0.000	0.007	0.001	0.011	0.003	n.a.	n.a.	0.025	0.005	0.042	0.002	0.021	0.004	0.031	0.005	0.036	0.003	0.061	0.006	0.088	0.007
p-Coumaric acid (pCd)	0.000	0.000	0.015	0.001	0.026	0.001	n.a.	n.a.	0.038	0.002	0.043	0.005	0.029	0.001	0.063	0.003	0.070	0.003	0.091	0.002	0.084	0.013
Ferulic acid (Fd)	0.000	0.000	0.012	0.002	0.022	0.005	n.a.	n.a.	0.032	0.002	0.030	0.015	0.035	0.010	0.056	0.008	0.051	0.008	0.082	0.007	0.051	0.020
$Ac/Al_{(V)}$	0.000	0.000	0.4	0.1	0.4	0.2	n.a.	n.a.	0.4	0.1	0.3	0.1	0.3	0.1	0.3	0.1	0.3	0.0	0.3	0.1	0.3	0.1
$Ac/Al_{(S)}$	0.000	0.000	0.2	0.1	0.3	0.1	n.a.	n.a.	0.3	0.1	0.4	0.3	0.4	0.1	0.3	0.0	0.3	0.0	0.3	0.0	0.6	0.2
$Ac/Al_{(V+S)}$	0.000	0.000	0.3	0.1	0.3	0.1	n.a.	n.a.	0.3	0.0	0.4	0.2	0.4	0.1	0.3	0.0	0.3	0.0	0.3	0.0	0.5	0.1
S/V	0.000	0.000	2.6	0.6	1.9	0.4	n.a.	n.a.	2.0	0.2	2.6	0.7	1.9	0.3	1.8	0.2	2.1	0.2	2.1	0.3	2.6	0.5
C/V	0.000	0.000	1.7	0.2	1.9	0.3	n.a.	n.a.	1.3	0.1	1.7	0.4	1.7	0.3	1.5	0.2	1.7	0.2	1.4	0.2	1.2	0.2

[a] The standard error is the analytical error of six lab replicates (years 0 and 3) or three lab replicates (years 6, 10, 15, 18) of one homogenized archived soil sample per treatment and sampling date.

n.a. sample not available

Lundgaard soil

Lignin monomers (C_4-derived)	Concentration/ mg g^{-1} soil																					
	control		low input										high input									
	0		3		6		10		15		18		3		6		10		15		18	
	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]
Vanillin (Vl)	0.000	0.000	0.004	0.001	0.011	0.001	n.a.	n.a.	0.029	0.002	0.027	0.001	0.012	0.002	0.034	0.002	0.035	0.002	0.057	0.004	0.061	0.003
Vanillic acid (Vd)	0.000	0.000	0.001	0.000	0.004	0.000	n.a.	n.a.	0.011	0.002	0.008	0.001	0.004	0.001	0.010	0.003	0.010	0.003	0.016	0.002	0.016	0.001
Acetovanillone (Vn)	0.000	0.000	0.001	0.000	0.002	0.000	n.a.	n.a.	0.008	0.000	0.008	0.000	0.004	0.001	0.008	0.001	0.008	0.001	0.014	0.002	0.018	0.002
Syringaldehyde (Sl)	0.000	0.000	0.010	0.002	0.017	0.004	n.a.	n.a.	0.049	0.003	0.071	0.002	0.019	0.003	0.054	0.005	0.054	0.005	0.096	0.005	0.124	0.005
Syringic acid (Sd)	0.000	0.000	0.002	0.001	0.005	0.002	n.a.	n.a.	0.019	0.001	0.021	0.002	0.008	0.001	0.021	0.003	0.019	0.001	0.035	0.005	0.041	0.003
Acetosyringone (Sn)	0.000	0.000	0.003	0.001	0.006	0.001	n.a.	n.a.	0.021	0.004	0.029	0.002	0.011	0.002	0.024	0.006	0.025	0.007	0.048	0.008	0.058	0.007
p-Coumaric acid (pCd)	0.000	0.000	0.004	0.001	0.009	0.001	n.a.	n.a.	0.026	0.001	0.027	0.002	0.011	0.001	0.033	0.001	0.034	0.004	0.049	0.009	0.043	0.010
Ferulic acid (Fd)	0.000	0.000	0.002	0.001	0.004	0.002	n.a.	n.a.	0.017	0.003	0.010	0.003	0.008	0.001	0.025	0.003	0.022	0.000	0.041	0.003	0.022	0.011
$Ac/Al_{(V)}$	0.000	0.000	0.4	0.1	0.4	0.0	n.a.	n.a.	0.4	0.1	0.3	0.0	0.3	0.1	0.3	0.1	0.3	0.1	0.3	0.0	0.3	0.0
$Ac/Al_{(S)}$	0.000	0.000	0.2	0.1	0.3	0.1	n.a.	n.a.	0.4	0.0	0.3	0.0	0.4	0.1	0.4	0.1	0.3	0.0	0.4	0.1	0.3	0.0
$Ac/Al_{(V+S)}$	0.000	0.000	0.2	0.1	0.3	0.1	n.a.	n.a.	0.4	0.0	0.3	0.0	0.4	0.1	0.3	0.0	0.3	0.0	0.3	0.0	0.3	0.0
S/V	0.000	0.000	2.3	0.6	1.6	0.3	n.a.	n.a.	1.9	0.1	2.8	0.1	1.9	0.3	1.9	0.2	1.8	0.2	2.0	0.2	2.4	0.1
C/V	0.000	0.000	1.0	0.3	0.8	0.1	n.a.	n.a.	0.9	0.1	0.9	0.1	1.0	0.1	1.1	0.1	1.0	0.1	1.0	0.1	0.7	0.2

[a] The standard error is the analytical error of six lab replicates (years 0 and 3) or three lab replicates (years 6, 10, 15, 18) of one homogenized archived soil sample per treatment and sampling date.

n.a. sample not available

Table 9 C_3-derived lignin monomers in soil samples.

Askov soil

Lignin monomers (C_3-derived)	Concentration/ mg g^{-1} soil																					
	control		low input										high input									
	0		3		6		10		15		18		3		6		10		15		18	
	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]
Vanillin (Vl)	0.108	0.002	0.100	0.005	0.081	0.009	n.a.	n.a.	0.071	0.005	0.078	0.005	0.110	0.004	0.091	0.014	0.081	0.012	0.073	0.014	0.081	0.006
Vanillic acid (Vd)	0.060	0.010	0.054	0.009	0.030	0.005	n.a.	n.a.	0.030	0.005	0.060	0.004	0.057	0.010	0.033	0.004	0.027	0.004	0.024	0.004	0.069	0.007
Acetovanillone (Vn)	0.044	0.003	0.040	0.004	0.029	0.003	n.a.	n.a.	0.026	0.001	0.034	0.002	0.041	0.003	0.032	0.002	0.028	0.001	0.024	0.001	0.039	0.003
Syringaldehyde (Sl)	0.092	0.001	0.074	0.007	0.070	0.003	n.a.	n.a.	0.059	0.001	0.073	0.025	0.092	0.009	0.071	0.002	0.059	0.004	0.050	0.010	0.076	0.055
Syringic acid (Sd)	0.068	0.009	0.061	0.011	0.036	0.008	n.a.	n.a.	0.031	0.006	0.038	0.011	0.062	0.010	0.045	0.007	0.035	0.006	0.045	0.010	0.054	0.002
Acetosyringone (Sn)	0.060	0.005	0.057	0.006	0.036	0.000	n.a.	n.a.	0.032	0.001	0.048	0.001	0.060	0.005	0.045	0.008	0.036	0.005	0.035	0.005	0.058	0.005
p-Coumaric acid (pCd)	0.031	0.002	0.029	0.003	0.018	0.002	n.a.	n.a.	0.015	0.003	0.026	0.002	0.033	0.005	0.033	0.004	0.024	0.008	0.027	0.008	0.052	0.010
Ferulic acid (Fd)	0.048	0.005	0.041	0.003	0.034	0.003	n.a.	n.a.	0.020	0.002	0.003	0.002	0.034	0.008	0.027	0.003	0.029	0.006	0.018	0.005	0.004	0.006
$Ac/Al_{(V)}$	0.6	0.1	0.5	0.1	0.4	0.1	n.a.	n.a.	0.4	0.1	0.8	0.1	0.5	0.1	0.4	0.1	0.3	0.1	0.3	0.1	0.8	0.1
$Ac/Al_{(S)}$	0.7	0.1	0.8	0.2	0.5	0.1	n.a.	n.a.	0.5	0.1	0.5	0.2	0.7	0.1	0.6	0.1	0.6	0.1	0.9	0.3	0.7	0.5
$Ac/Al_{(V+S)}$	0.6	0.1	0.7	0.1	0.4	0.1	n.a.	n.a.	0.5	0.1	0.7	0.1	0.6	0.1	0.5	0.1	0.4	0.1	0.6	0.1	0.8	0.3
S/V	1.0	0.1	1.0	0.1	1.0	0.1	n.a.	n.a.	1.0	0.1	0.9	0.2	1.0	0.1	1.0	0.1	1.0	0.1	1.1	0.2	1.0	0.3
C/V	0.4	0.0	0.4	0.0	0.4	0.0	n.a.	n.a.	0.3	0.0	0.2	0.0	0.3	0.0	0.4	0.0	0.4	0.1	0.4	0.1	0.3	0.1

[a] The standard error is the analytical error of six lab replicates (years 0 and 3) or three lab replicates (years 6, 10, 15, 18) of one homogenized archived soil sample per treatment and sampling date.

n.a. sample not available

Lundgaard soil

Lignin monomers (C_3-derived)	Concentration/ mg g^{-1} soil																					
	control		low input										high input									
	0		3		6		10		15		18		3		6		10		15		18	
	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]
Vanillin (Vl)	0.060	0.001	0.052	0.003	0.044	0.002	n.a.	n.a.	0.035	0.002	0.036	0.002	0.055	0.002	0.046	0.002	0.040	0.005	0.039	0.002	0.045	0.004
Vanillic acid (Vd)	0.028	0.003	0.025	0.004	0.017	0.003	n.a.	n.a.	0.014	0.003	0.022	0.002	0.025	0.002	0.017	0.001	0.013	0.002	0.014	0.001	0.031	0.003
Acetovanillone (Vn)	0.020	0.001	0.018	0.001	0.015	0.001	n.a.	n.a.	0.012	0.001	0.013	0.001	0.019	0.001	0.016	0.001	0.014	0.002	0.016	0.001	0.018	0.002
Syringaldehyde (Sl)	0.055	0.001	0.043	0.002	0.039	0.005	n.a.	n.a.	0.034	0.004	0.019	0.003	0.048	0.003	0.040	0.006	0.039	0.006	0.040	0.006	0.028	0.008
Syringic acid (Sd)	0.036	0.002	0.032	0.003	0.023	0.002	n.a.	n.a.	0.025	0.003	0.024	0.005	0.032	0.002	0.029	0.002	0.026	0.003	0.031	0.002	0.042	0.008
Acetosyringone (Sn)	0.033	0.003	0.029	0.003	0.023	0.001	n.a.	n.a.	0.020	0.002	0.022	0.002	0.029	0.002	0.029	0.005	0.024	0.004	0.024	0.006	0.029	0.002
p-Coumaric acid (pCd)	0.012	0.001	0.011	0.001	0.011	0.002	n.a.	n.a.	0.012	0.002	0.019	0.002	0.014	0.001	0.018	0.005	0.019	0.005	0.023	0.004	0.052	0.010
Ferulic acid (Fd)	0.021	0.002	0.018	0.003	0.019	0.001	n.a.	n.a.	0.010	0.003	0.012	0.005	0.018	0.003	0.019	0.005	0.021	0.004	0.017	0.003	0.018	0.011
$Ac/Al_{(V)}$	0.5	0.0	0.5	0.1	0.4	0.1	n.a.	n.a.	0.4	0.1	0.6	0.1	0.4	0.0	0.4	0.0	0.3	0.1	0.4	0.0	0.7	0.1
$Ac/Al_{(S)}$	0.7	0.0	0.7	0.1	0.6	0.1	n.a.	n.a.	0.7	0.1	1.3	0.3	0.7	0.1	0.7	0.1	0.7	0.1	0.8	0.1	1.5	0.5
$Ac/Al_{(V+S)}$	0.6	0.0	0.6	0.1	0.5	0.0	n.a.	n.a.	0.6	0.1	0.8	0.1	0.5	0.0	0.5	0.0	0.5	0.1	0.6	0.1	1.0	0.2
S/V	1.1	0.0	1.1	0.1	1.1	0.1	n.a.	n.a.	1.3	0.1	0.9	0.1	1.1	0.1	1.2	0.1	1.3	0.2	1.4	0.1	1.1	0.1
C/V	0.3	0.0	0.3	0.0	0.4	0.0	n.a.	n.a.	0.4	0.1	0.4	0.1	0.3	0.0	0.5	0.1	0.6	0.1	0.6	0.1	0.8	0.2

[a] The standard error is the analytical error of six lab replicates (years 0 and 3) or three lab replicates (years 6, 10, 15, 18) of one homogenized archived soil sample per treatment and sampling date.

n.a. sample not available

Table 10 $\delta^{13}C$ values of SOC and lignin carbon in Askov and Lundgaard soil samples.

Treatment	Year	$\delta^{13}C$ SOC/ ‰ V-PDB				$\delta^{13}C$ VSC/ ‰ V-PDB			
		Askov soil		Lundgaard soil		Askov soil		Lundgaard soil	
		Mean	SE [a]	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]
control	0	-27.8	0.0	-26.3	0.4	-32.6	0.7	-32.4	0.5
low input	3	-27.4	0.1	-26.7	0.0	-29.8	0.6	-30.6	0.5
	6	-26.8	0.0	-26.1	0.0	-28.9	0.6	-28.6	1.0
	10	no sample available				no sample available		no sample available	
	15	-25.9	0.0	-24.4	0.1	-25.5	0.5	-23.4	0.7
	18	-25.6	0.0	-24.0	0.2	-24.7	0.5	-21.7	0.8
high input	3	-26.4	0.1	-25.8	0.1	-27.9	0.4	-28.0	0.6
	6	-26.0	0.0	-23.4	0.0	-24.9	0.4	-23.8	0.7
	10	-25.7	0.1	-23.7	0.1	-24.1	0.5	-23.4	0.7
	15	-24.3	0.1	-21.8	0.2	-21.9	1.0	-21.3	0.2
	18	-23.7	0.0	-21.5	0.1	-21.7	0.8	-20.6	0.2

[a] The standard error is the analytical error of two lab replicates of one homogenized archived soil sample per treatment and sampling date.

Table 11 Lignin monomers in Askov plant samples.

Lignin monomers (total)	Concentration/ mg g^{-1} plant dry matter			
	Barley straw (1989-1992)		Maize stover (2001)	
	Mean	SE [a]	Mean	SE [a]
Vanillin (Vl)	11.3	1.0	7.1	0.3
Vanillic acid (Vd)	2.3	1.2	1.4	0.1
Acetovanillone (Vn)	1.7	0.7	1.1	0.4
Syringaldehyde (Sl)	10.3	0.1	7.6	0.1
Syringc acid (Sd)	4.3	0.7	3.2	0.2
Acetosyringone (Sn)	6.2	0.3	5.4	0.3
p-Coumaric acid (pCd)	3.6	0.2	8.1	0.3
Ferulic acid (Fd)	4.5	0.7	5.1	0.5
$Ac/Al_{(V)}$	0.2	0.1	0.2	0.0
$Ac/Al_{(S)}$	0.4	0.1	0.4	0.0
$Ac/Al_{(V+S)}$	0.3	0.1	0.3	0.0
S/V	1.4	0.2	1.7	0.1
C/V	0.5	0.1	1.4	0.1

[a] The standard error is the analytical error of three lab replicates of one homogenized sample.

Table 12 Characteristics of above-ground plant biomass input.

Plant material	OC / mg g^{-1} plant dry matter		VSC / mg g^{-1} plant dry matter		/ mg g^{-1} OC		C_{VSC} / mg g^{-1} plant dry matter		/ mg g^{-1} OC		δ^{13}C OC / ‰ V-PDB		δ^{13}C VSC / ‰ V-PDB	
	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]
Barley straw (1989-1992)	430.6	1.3	44.2	2.0	102.6	4.7	27.0	1.2	62.6	2.8	-25.4	0.6	-18.9	0.1
Maize stover (2001)	421.9	1.1	39.0	0.9	92.5	2.1	24.0	0.5	57.0	1.3	-12.2	0.3	-35.7	0.5

[a] The standard error is the analytical error of three lab replicates of one homogenized sample.

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Table 13 Soil organic carbon concentrations in Cadriano soil samples.

Treatment	Crop	Year	total SOC /mg g^{-1} soil		C_4-SOC /mg g^{-1} soil		C_3-SOC /mg g^{-1} soil	
			Mean	SE [a]	Mean	SE [a]	Mean	SE [a]
non-fertilized	Wheat	7	8.0		0.0		8.0	
non-fertilized	Maize	7	7.6	0.3	0.0	0.1	7.6	0.1
		14	7.4	0.9	1.1	0.6	6.2	0.6
		19	7.4	0.6	0.8	0.2	6.6	0.2
		31	7.0	0.3	1.0	0.2	6.0	0.2
		36	7.2		0.9		6.4	
fertilized	Wheat	7	8.7		0.0		8.7	
fertilized	Maize	7	7.2	0.8	0.3	0.3	6.9	0.3
		14	7.4	1.2	0.8	0.2	6.6	0.2
		19	7.1	0.4	0.7	0.0	6.4	0.0
		31	7.0	0.8	1.1	0.2	6.0	0.2
		36	8.3		2.2		6.1	

[a] The standard error was calculated from two field replicate samples from the soil archive (no replicates available for year 7 Wheat and year 36 Maize).

Table 14 VSC-lignin concentrations in Cadriano soil samples.

Treatment	Crop	Year	total VSC / mg g^{-1} soil		C_4-VSC / mg g^{-1} soil		C_3-VSC / mg g^{-1} soil	
			Mean	SE [a]	Mean	SE [a]	Mean	SE [a]
non-fertilized	Wheat	7	0.200		0.000		0.200	
non-fertilized	Maize	7	0.173	0.013	0.013	0.003	0.161	0.015
		14	0.161	0.018	0.023	0.007	0.140	0.019
		19	0.215	0.046	0.041	0.011	0.172	0.043
		31	0.172	0.005	0.052	0.004	0.123	0.003
		36	0.114		0.046		0.066	
fertilized	Wheat	7	0.218		0.000		0.218	
fertilized	Maize	7	0.143	0.006	0.016	0.002	0.128	0.008
		14	0.198	0.015	0.020	0.001	0.178	0.016
		19	0.196	0.005	0.037	0.004	0.157	0.005
		31	0.169	0.009	0.040	0.003	0.132	0.007
		36	0.164		0.048		0.117	

[a] The standard error was calculated from two field replicate samples from the soil archive (no replicates available for year 7 Wheat and year 36 Maize).

Table 15 VSC-lignin concentrations in SOC of Cadriano soil samples.

Treatment	Crop	Year	total VSC / mg g^{-1} SOC		C_4-VSC / mg g^{-1} SOC		C_3-VSC / mg g^{-1} SOC	
			Mean	SE [a]	Mean	SE [a]	Mean	SE [a]
non-fertilized	Wheat	7	24.9		0.0		24.9	
non-fertilized	Maize	7	22.8	2.1	1.7	0.4	21.3	2.2
		14	21.8	3.5	3.2	1.1	18.9	3.4
		19	29.0	6.7	5.6	1.6	23.3	6.2
		31	24.5	1.1	7.4	0.6	17.4	0.8
		36	15.8		6.4		9.1	
fertilized	Wheat	7						
fertilized	Maize	7	19.9	2.5	2.2	0.4	17.7	2.3
		14	26.8	4.8	2.7	0.5	24.1	4.4
		19	27.4	1.6	5.1	0.6	22.0	1.3
		31	24.1	3.0	5.7	0.7	18.7	2.4
		36	19.8		5.8	0.0	14.1	

[a] The standard error was calculated from two field replicate samples from the soil archive (no replicates available for year 7 Wheat and year 36 Maize).

Table 16 Lignin carbon (C_{VSC}) concentrations in Cadriano soil samples.

Treatment	Crop	Year	C_{VSC} total /mg g^{-1} soil		$C_{C4\text{-}VSC}$ /mg g^{-1} soil		$C_{C3\text{-}VSC}$ /mg g^{-1} soil	
			Mean	SE [a]	Mean	SE [a]	Mean	SE [a]
non-fertilized	Wheat	7	0.121		0.000		0.121	
non-fertilized	Maize	7	0.105	0.019	0.007	0.001	0.097	0.020
		14	0.098	0.024	0.013	0.000	0.085	0.025
		19	0.130	0.068	0.027	0.002	0.103	0.066
		31	0.104	0.004	0.031	0.003	0.074	0.000
		36	0.069		0.028		0.040	
fertilized	Wheat	7	0.132		0.000		0.132	
fertilized	Maize	7	0.087	0.008	0.009	0.003	0.077	0.010
		14	0.120	0.024	0.012	0.001	0.107	0.025
		19	0.118	0.005	0.023	0.001	0.095	0.006
		31	0.103	0.014	0.024	0.003	0.078	0.011
		36	0.100		0.029		0.071	

[a] The standard error was calculated from two field replicate samples from the soil archive (no replicates available for year 7 Wheat and year 36 Maize).

Table 17 Lignin carbon (C_{VSC}) concentrations in SOC of Cadriano soil samples.

Treatment	Crop	Year	C_{VSC} total /mg g^{-1} SOC		$C_{C4\text{-}VSC}$ /mg g^{-1} SOC		$C_{C3\text{-}VSC}$ /mg g^{-1} SOC	
			Mean	SE [a]	Mean	SE [a]	Mean	SE [a]
non-fertilized	Wheat	7	15.0		0.0		15.0	
non-fertilized	Maize	7	13.7	1.9	1.0	0.2	12.8	2.1
		14	13.1	1.7	1.8	0.3	11.3	2.0
		19	17.0	7.8	3.7	0.0	13.3	7.8
		31	14.9	1.1	4.4	0.7	10.5	0.4
		36	9.6		3.9		5.5	
fertilized	Wheat	7	15.2		0.0		15.2	
fertilized	Maize	7	12.3	2.5	1.3	0.2	11.0	2.7
		14	16.1	0.7	1.7	0.4	14.4	1.1
		19	16.6	1.6	3.3	0.0	13.4	1.6
		31	14.6	0.3	3.5	0.0	11.1	0.3
		36	12.0		3.5		8.6	

[a] The standard error was calculated from two field replicate samples from the soil archive (no replicates available for year 7 Wheat and year 36 Maize).

Table 18 Lignin monomer concentrations in Cadriano soil samples.

Cadriano, non-fertilized

Lignin monomers (total)	Concentration/ mg g^{-1} soil											
	Wheat non-fertilized		Maize non-fertilized									
	7		7		14		19		31		36	
	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]
Vanillin (Vl)	0.048		0.041	0.010	0.042	0.014	0.046	0.026	0.037	0.001	0.023	
Vanillic acid (Vd)	0.025		0.022	0.004	0.018	0.005	0.021	0.008	0.017	0.001	0.011	
Acetovanillone (Vn)	0.013		0.015	0.003	0.014	0.003	0.016	0.007	0.013	0.000	0.008	
Syringaldehyde (Sl)	0.044		0.040	0.005	0.036	0.007	0.052	0.029	0.042	0.001	0.029	
Syringic acid (Sd)	0.026		0.020	0.002	0.017	0.001	0.026	0.011	0.020	0.001	0.013	
Acetosyringone (Sn)	0.021		0.020	0.004	0.019	0.004	0.028	0.015	0.020	0.000	0.013	
p-Coumaric acid (pCd)	0.015		0.012	0.002	0.015	0.003	0.023	0.013	0.020	0.003	0.014	
Ferulic acid (Fd)	0.006		0.001	0.001	0.001	0.001	0.003	0.003	0.003	0.003	0.002	
$Ac/Al_{(V)}$	0.5		0.5	0.2	0.4	0.2	0.5	0.3	0.5	0.0	0.5	
$Ac/Al_{(S)}$	0.6		0.5	0.1	0.5	0.1	0.5	0.4	0.5	0.0	0.5	
$Ac/Al_{(V+S)}$	0.6		0.5	0.1	0.4	0.1	0.5	0.2	0.5	0.0	0.5	
S/V	1.1		1.0	0.2	1.0	0.2	1.3	0.6	1.2	0.0	1.3	
C/V	0.2		0.2	0.0	0.2	0.1	0.3	0.2	0.3	0.1	0.4	

[a] The standard error was calculated from two field replicate samples from the soil archive (no replicates available for year 7 Wheat and year 36 Maize).

Cadriano, fertilized

Lignin monomers (total)	Concentration/ mg g^{-1} soil											
	Wheat fertilized		Maize fertilized									
	7		7		14		19		31		36	
	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]
Vanillin (Vl)	0.049		0.033	0.005	0.043	0.007	0.042	0.004	0.033	0.003	0.030	
Vanillic acid (Vd)	0.025		0.020	0.002	0.022	0.004	0.021	0.001	0.017	0.001	0.015	
Acetovanillone (Vn)	0.018		0.013	0.001	0.016	0.002	0.016	0.000	0.013	0.001	0.011	
Syringaldehyde (Sl)	0.045		0.032	0.002	0.045	0.009	0.045	0.002	0.041	0.005	0.041	
Syringic acid (Sd)	0.030		0.018	0.001	0.028	0.007	0.029	0.001	0.023	0.002	0.022	
Acetosyringone (Sn)	0.025		0.017	0.001	0.025	0.006	0.023	0.000	0.021	0.003	0.020	
p-Coumaric acid (pCd)	0.020		0.011	0.000	0.016	0.003	0.016	0.001	0.019	0.004	0.022	
Ferulic acid (Fd)	0.007		0.000	0.000	0.004	0.003	0.004	0.000	0.003	0.003	0.003	
$Ac/Al_{(V)}$	0.5		0.6	0.1	0.5	0.1	0.5	0.1	0.5	0.0	0.5	
$Ac/Al_{(S)}$	0.7		0.6	0.1	0.6	0.2	0.6	0.0	0.6	0.1	0.5	
$Ac/Al_{(V+S)}$	0.6		0.6	0.1	0.6	0.1	0.6	0.0	0.5	0.1	0.5	
S/V	1.1		1.0	0.1	1.2	0.2	1.2	0.1	1.4	0.1	1.5	
C/V	0.3		0.2	0.0	0.2	0.1	0.3	0.0	0.4	0.1	0.4	

[a] The standard error was calculated from two field replicate samples from the soil archive (no replicates available for year 7 Wheat and year 36 Maize).

Table 19 C_4-derived lignin monomers in SOC of Cadriano soil samples.

Cadriano, non-fertilized

Lignin monomers (C_4-derived)	Concentration/ mg g^{-1} soil											
	Wheat non-fertilized		Maize non-fertilized									
	7		7		14		19		31		36	
	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]
Vanillin (Vl)	0.000	0.000	0.001	0.001	0.007	0.001	0.010	0.002	0.012	0.001	0.011	
Vanillic acid (Vd)	0.000	0.000	0.000	0.002	0.000	0.005	0.000	0.009	0.000	0.002	0.000	
Acetovanillone (Vn)	0.000	0.000	0.000	0.001	0.001	0.000	0.002	0.000	0.002	0.001	0.002	
Syringaldehyde (Sl)	0.000	0.000	0.002	0.001	0.004	0.001	0.015	0.004	0.021	0.001	0.022	
Syringic acid (Sd)	0.000	0.000	0.001	0.001	0.005	0.000	0.006	0.000	0.007	0.000	0.006	
Acetosyringone (Sn)	0.000	0.000	0.002	0.000	0.000	0.005	0.001	0.005	0.000	0.002	0.000	
p-Coumaric acid (pCd)	0.000	0.000	0.003	0.001	0.004	0.000	0.007	0.002	0.007	0.001	0.006	
Ferulic acid (Fd)	0.000	0.000	0.002		0.003		0.001		0.004		0.000	
$Ac/Al_{(V)}$	0.0	0.0	0.0	2.3	0.0	0.8	0.0	0.9	0.0	0.2	0.0	
$Ac/Al_{(S)}$	0.0	0.0	0.6	0.4	1.3	0.2	0.4	0.1	0.3	0.0	0.3	
$Ac/Al_{(V+S)}$	0.0	0.0	0.5	0.7	0.5	0.5	0.2	0.4	0.2	0.1	0.2	
S/V	0.0	0.0	5.5	11.0	1.0	0.9	1.8	1.5	2.0	0.4	2.1	
C/V	0.0	0.0	5.0	9.9	0.8	0.5	0.7	0.6	0.8	0.2	0.4	

[a] The standard error was calculated from two field replicate samples from the soil archive (no replicates available for year 7 Wheat and year 36 Maize).

Cadriano, fertilized

Lignin monomers (C_4-derived)	Concentration/ mg g^{-1} soil											
	Wheat fertilized		Maize fertilized									
	7		7		14		19		31		36	
	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]
Vanillin (Vl)	0.000	0.000	0.003	0.001	0.008	0.000	0.009	0.001	0.010	0.002	0.012	
Vanillic acid (Vd)	0.000	0.000	0.002	0.001	0.000	0.002	0.000	0.009	0.000	0.001	0.000	
Acetovanillone (Vn)	0.000	0.000	0.000	0.000	0.001	0.000	0.002	0.000	0.002	0.001	0.002	
Syringaldehyde (Sl)	0.000	0.000	0.005	0.000	0.008	0.001	0.016	0.002	0.022	0.000	0.025	
Syringic acid (Sd)	0.000	0.000	0.001	0.001	0.001	0.001	0.003	0.001	0.004	0.001	0.006	
Acetosyringone (Sn)	0.000	0.000	0.002	0.001	0.000	0.002	0.005	0.003	0.000	0.005	0.000	
p-Coumaric acid (pCd)	0.000	0.000	0.002	0.001	0.001	0.001	0.002	0.000	0.002	0.000	0.002	
Ferulic acid (Fd)	0.000	0.000			0.000	0.000	0.000	0.001			0.001	
$Ac/Al_{(V)}$	0.0	0.0	0.7	0.4	0.0	0.2	0.0	1.0	0.0	0.1	0.0	
$Ac/Al_{(S)}$	0.0	0.0	0.3	0.2	0.2	0.1	0.2	0.1	0.2	0.0	0.3	
$Ac/Al_{(V+S)}$	0.0	0.0	0.4	0.2	0.1	0.1	0.1	0.4	0.1	0.0	0.2	
S/V	0.0	0.0	1.6	0.5	1.1	0.3	2.1	1.8	2.3	0.7	2.3	
C/V	0.0	0.0	0.3	0.2	0.1	0.1	0.2	0.2	0.2	0.0	0.2	

[a] The standard error was calculated from two field replicate samples from the soil archive (no replicates available for year 7 Wheat and year 36 Maize).

Table 20 C_3-derived lignin monomers in SOC of Cadriano soil samples.

Cadriano, non-fertilized

Lignin monomers (C_3-derived)	Concentration/ mg g^{-1} soil											
	Wheat non-fertilized		Maize non-fertilized									
	7		7		14		19		31		36	
	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]
Vanillin (Vl)	0.048		0.041	0.011	0.035	0.016	0.036	0.024	0.025	0.002	0.012	
Vanillic acid (Vd)	0.025		0.022	0.004	0.018	0.005	0.019	0.010	0.017	0.001	0.011	
Acetovanillone (Vn)	0.013		0.015	0.004	0.012	0.004	0.014	0.007	0.011	0.001	0.006	
Syringaldehyde (Sl)	0.044		0.038	0.006	0.032	0.008	0.037	0.025	0.021	0.000	0.008	
Syringic acid (Sd)	0.026		0.019	0.003	0.012	0.001	0.020	0.011	0.013	0.000	0.007	
Acetosyringone (Sn)	0.021		0.018	0.004	0.019	0.004	0.025	0.018	0.020	0.000	0.013	
p-Coumaric acid (pCd)	0.015		0.009	0.001	0.011	0.003	0.016	0.011	0.013	0.002	0.009	
Ferulic acid (Fd)	0.006						0.005		0.002		0.000	
$Ac/Al_{(V)}$	0.5		0.5	0.2	0.5	0.3	0.5	0.4	0.7	0.1	0.9	
$Ac/Al_{(S)}$	0.6		0.5	0.1	0.4	0.1	0.5	0.5	0.6	0.0	1.0	
$Ac/Al_{(V+S)}$	0.6		0.5	0.1	0.4	0.1	0.5	0.3	0.7	0.0	0.9	
S/V	1.1		1.0	0.2	1.0	0.3	1.2	0.7	1.0	0.0	0.9	
C/V	0.2		0.1	0.0	0.2	0.1	0.3	0.2	0.3	0.0	0.3	

[a] The standard error was calculated from two field replicate samples from the soil archive (no replicates available for year 7 Wheat and year 36 Maize).

Cadriano, fertilized

Lignin monomers (C_3-derived)	Concentration/ mg g^{-1} soil											
	Wheat fertilized		Maize fertilized									
	7		7		14		19		31		36	
	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]
Vanillin (Vl)	0.049		0.030	0.006	0.035	0.006	0.032	0.002	0.023	0.000	0.019	
Vanillic acid (Vd)	0.025		0.017	0.003	0.022	0.004	0.019	0.003	0.017	0.001	0.015	
Acetovanillone (Vn)	0.018		0.013	0.001	0.015	0.003	0.014	0.001	0.011	0.001	0.009	
Syringaldehyde (Sl)	0.045		0.027	0.002	0.036	0.009	0.030	0.000	0.019	0.005	0.016	
Syringic acid (Sd)	0.030		0.017	0.002	0.026	0.007	0.026	0.001	0.018	0.001	0.015	
Acetosyringone (Sn)	0.025		0.015	0.002	0.025	0.006	0.019	0.002	0.021	0.003	0.020	
p-Coumaric acid (pCd)	0.020		0.009	0.000	0.016	0.004	0.014	0.002	0.017	0.004	0.021	
Ferulic acid (Fd)	0.007		0.000		0.003		0.003		0.006		0.002	
$Ac/Al_{(V)}$	0.5		0.6	0.2	0.6	0.2	0.6	0.1	0.7	0.0	0.8	
$Ac/Al_{(S)}$	0.7		0.6	0.1	0.7	0.3	0.9	0.0	0.9	0.3	1.0	
$Ac/Al_{(V+S)}$	0.6		0.6	0.1	0.7	0.2	0.7	0.1	0.8	0.1	0.9	
S/V	1.1		1.0	0.1	1.2	0.2	1.1	0.1	1.1	0.1	1.2	
C/V	0.3		0.2	0.0	0.3	0.1	0.3	0.0	0.4	0.1	0.5	

[a] The standard error was calculated from two field replicate samples from the soil archive (no replicates available for year 7 Wheat and year 36 Maize).

Table 21 $\delta^{13}C$ values of SOC in Cadriano soils.

Treatment	Crop	Year	$\delta^{13}C$ SOC/ ‰ V-PDB	
			Mean	SE [a]
non-fertilized	Wheat	7	-24.7	
non-fertilized	Maize	7	-24.7	0.2
		14	-23.6	0.0
		19	-23.3	0.4
		31	-22.7	0.3
		36	-23.1	
fertilized	Wheat	7	-24.8	
fertilized	Maize	7	-24.2	0.5
		14	-23.3	0.2
		19	-23.4	0.0
		31	-22.74	0.2
		36	-21.18	

[a] The standard error was calculated from two field replicate samples (no replicates available for year 7 Wheat and year 36 Maize).

Table 22 Lignin monomers in Cadriano plant samples.

Lignin monomers (total)	Concentration/ mg g^{-1} plant dry matter							
	Wheat straw (1980)[a]				Maize stover (1973-1997)[b]			
	non-fertilized		fertilized		non-fertilized		fertilized	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Vanillin (Vl)	16.7	0.1	16.3	0.5	8.9	0.9	8.4	0.2
Vanillic acid (Vd)	2.0	0.2	2.0	0.2	1.1	0.2	1.1	0.1
Acetovanillone (Vn)	3.0	0.1	2.9	0.1	1.7	0.1	1.6	0.1
Syringaldehyde (Sl)	15.0	0.1	15.9	0.5	8.5	3.4	8.5	0.5
Syringc acid (Sd)	4.4	0.4	5.0	0.2	2.6	0.3	2.3	0.3
Acetosyringone (Sn)	7.6	0.1	7.8	0.1	3.6	1.3	4.6	0.2
p-Coumaric acid (pCd)	4.4	0.0	5.2	0.1	11.7	2.7	8.2	0.9
Ferulic acid (Fd)	5.0	0.5	5.1	0.2	5.0	0.7	4.9	0.3
$Ac/Al_{(V)}$	0.1	0.0	0.1	0.0	0.1	0.0	0.1	0.0
$Ac/Al_{(S)}$	0.3	0.0	0.3	0.0	0.3	0.1	0.3	0.0
$Ac/Al_{(V+S)}$	0.2	0.0	0.2	0.0	0.2	0.0	0.2	0.0
S/V	1.2	0.0	1.3	0.0	1.3	0.3	1.4	0.1
C/V	0.4	0.0	0.5	0.0	1.4	0.3	1.2	0.1

[a] The standard error was calculated from three analytical replicates of one homogenized straw sample (year 1980).

[b] The standard error was calculated from three analytical replicates of homogenized stover samples (years 1973, 1980, 1985, 1997).

Table 23 Characteristics of above-ground plant biomass input.

Plant material	OC / mg g^{-1} plant dry matter		VSC / mg g^{-1} plant dry matter		/ mg g^{-1} OC		C_{VSC} / mg g^{-1} plant dry matter		/ mg g^{-1} OC		δ^{13}C OC / ‰ V-PDB		δ^{13}C VSC / ‰ V-PDB	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Wheat straw (1980)[a]														
non-fertilized	389.0	2.0	58.0	0.9	149.1	2.4	35.5	0.5	91.3	1.4	-25.8	0.1	-32.9	0.6
fertilized	397.8	6.8	60.3	1.4	151.7	4.4	36.9	0.8	92.8	2.7	-26.4	0.0	-33.1	0.5
Maize stover (1973-1997)[b]														
non-fertilized	397.0	6.3	43.1	6.0	108.4	15.2	26.8	4.3	67.5	10.9	-12.9	0.2	-17.2	0.8
fertilized	423.1	3.2	39.6	1.4	93.5	3.5	24.5	1.0	57.9	2.5	-12.5	0.2	-15.3	0.8

[a] The standard error was calculated from three analytical replicates of one homogenized straw sample (year 1980).

[b] The standard error was calculated from three analytical replicates of homogenized stover samples (years 1973, 1980, 1985, 1997).

Data supplement to manuscript III

Table 24 Lignin (VSC) concentrations in fractions of the Askov soil.

Fraction		Lignin (VSC) concentration / mg g^{-1} fraction				/ mg g^{-1} soil			
		1988		2006		1988		2006	
		Mean	SE[1]	Mean	SE[1]	Mean	SE[2]	Mean	SE[2]
1	bulk soil < 2000 µm	0.5	± 0.0	1.1	± 0.0	0.539	± 0.017	1.074	± 0.030
1L	< 1.85 g cm^{-3}	10.4	± 0.7	18.1	± 0.4	0.046	± 0.003	0.255	± 0.005
1M	1.85 - 2.25 g cm^{-3}	2.4	± 0.1	4.4	± 0.1	0.128	± 0.003	0.149	± 0.002
1H	> 2.25 g cm^{-3}	0.3	± 0.0	0.6	± 0.0	0.282	± 0.006	0.555	± 0.006
2	size fraction 250 - 2000 µm	0.4	± 0.0	0.9	± 0.0	0.269	± 0.014	0.610	± 0.024
2L	< 1.85 g cm^{-3}	12.8	± 0.3	22.6	± 0.8	0.034	± 0.001	0.189	± 0.008
2M	1.85 - 2.25 g cm^{-3}	2.3	± 0.0	3.6	± 0.1	0.064	± 0.002	0.080	± 0.003
2H	> 2.25 g cm^{-3}	0.3	± 0.0	0.4	± 0.0	0.148	± 0.006	0.281	± 0.014
3	size fraction 20 - 250 µm	0.6	± 0.0	0.8	± 0.0	0.195	± 0.006	0.224	± 0.007
3L	< 1.85 g cm^{-3}	6.8	± 0.1	11.2	± 0.2	0.011	± 0.000	0.011	± 0.000
3M	1.85 - 2.25 g cm^{-3}	2.3	± 0.0	2.1	± 0.1	0.090	± 0.003	0.046	± 0.002
3H	> 2.25 g cm^{-3}	0.2	± 0.0	0.3	± 0.0	0.077	± 0.003	0.079	± 0.005
4	size fraction 2 - 20 µm	0.8	± 0.0	1.1	± 0.0	0.013	± 0.000	0.016	± 0.000
5	size fraction < 2 µm	0.8	± 0.1	1.3	± 0.0	0.001	± 0.000	0.001	± 0.000

[1] SE Standard error of N = 3 analytical replicates of lignin extraction from the fraction, each analytical replicate is measured in triplicate at the GC-FID.
[2] SE Standard error, error propagation calculation included

Table 25 Lignin carbon (C_{VSC}) concentrations in fractions of the Askov soil.

Fraction		Lignin (C_{VSC}) concentration / mg g^{-1} fraction				/ mg g^{-1} soil			
		1988		2006		1988		2006	
		Mean	SE[1]	Mean	SE[1]	Mean	SE[2]	Mean	SE[2]
1	bulk soil < 2000 µm	0.3	± 0.0	0.7	± 0.1	0.326	± 0.010	0.665	± 0.052
1L	< 1.85 g cm^{-3}	6.4	± 0.4	11.2	± 1.4	0.028	± 0.002	0.157	± 0.020
1M	1.85 - 2.25 g cm^{-3}	1.5	± 0.0	2.7	± 0.3	0.078	± 0.002	0.092	± 0.011
1H	> 2.25 g cm^{-3}	0.2	± 0.0	0.4	± 0.0	0.170	± 0.004	0.344	± 0.041
2	size fraction 250 - 2000 µm	0.3	± 0.0	0.6	± 0.0	0.162	± 0.008	0.376	± 0.023
2L	< 1.85 g cm^{-3}	7.8	± 0.2	14.0	± 1.4	0.021	± 0.000	0.117	± 0.011
2M	1.85 - 2.25 g cm^{-3}	1.4	± 0.0	2.2	± 0.2	0.039	± 0.001	0.049	± 0.005
2H	> 2.25 g cm^{-3}	0.2	± 0.0	0.3	± 0.0	0.089	± 0.003	0.172	± 0.020
3	size fraction 20 - 250 µm	0.3	± 0.0	0.5	± 0.0	0.118	± 0.002	0.138	± 0.008
3L	< 1.85 g cm^{-3}	4.1	± 0.1	6.9	± 0.4	0.007	± 0.000	0.007	± 0.000
3M	1.85 - 2.25 g cm^{-3}	1.4	± 0.0	1.3	± 0.1	0.055	± 0.001	0.028	± 0.002
3H	> 2.25 g cm^{-3}	0.2	± 0.0	0.2	± 0.0	0.046	± 0.001	0.048	± 0.004
4	size fraction 2 - 20 µm	0.5	± 0.0	0.7	± 0.0	0.008	± 0.000	0.010	± 0.000
5	size fraction < 2 µm	0.5	± 0.0	0.8	± 0.1	0.000	± 0.000	0.001	± 0.000

[1] SE Standard error of N = 3 analytical replicates of lignin extraction from the fraction, each analytical replicate is measured in triplicate at the GC-FID.
[2] SE Standard error, error propagation calculation included

Table 26 C_3-derived lignin carbon in fractions of the Askov soil.

Fraction		C3-derived lignin (C3-C_{VSC}) concentration / mg g^{-1} fraction				/ mg g^{-1} soil			
		1988		2006		1988		2006	
		Mean	SE[1]	Mean	SE[1]	Mean	SE[2]	Mean	SE[2]
1	bulk soil < 2000 µm	0.3	± 0.0	0.3	± 0.0	0.326	± 0.010	0.287	± 0.037
1L	< 1.85 g cm^{-3}	6.4	± 0.4	2.4	± 1.0	0.028	± 0.002	0.034	± 0.014
1M	1.85 - 2.25 g cm^{-3}	1.5	± 0.0	1.0	± 0.2	0.078	± 0.002	0.034	± 0.007
1H	> 2.25 g cm^{-3}	0.2	± 0.0	0.2	± 0.0	0.170	± 0.004	0.160	± 0.029
2	size fraction 250 - 2000 µm	0.3	± 0.0	0.2	± 0.0	0.162	± 0.008	0.141	± 0.016
2L	< 1.85 g cm^{-3}	7.8	± 0.2	2.3	± 1.0	0.021	± 0.000	0.020	± 0.008
2M	1.85 - 2.25 g cm^{-3}	1.4	± 0.0	0.3	± 0.2	0.039	± 0.001	0.006	± 0.004
2H	> 2.25 g cm^{-3}	0.2	± 0.0	0.0	± 0.0	0.089	± 0.003	0.029	± 0.014
3	size fraction 20 - 250 µm	0.3	± 0.0	0.2	± 0.0	0.118	± 0.002	0.064	± 0.006
3L	< 1.85 g cm^{-3}	4.1	± 0.1	1.5	± 0.3	0.007	± 0.000	0.001	± 0.000
3M	1.85 - 2.25 g cm^{-3}	1.4	± 0.0	0.3	± 0.1	0.055	± 0.001	0.006	± 0.001
3H	> 2.25 g cm^{-3}	0.2	± 0.0	0.0	± 0.0	0.046	± 0.001	0.010	± 0.003
4	size fraction 2 - 20 µm	0.5	± 0.0	0.4	± 0.0	0.008	± 0.000	0.006	± 0.000
5	size fraction < 2 µm	0.5	± 0.0	0.3	± 0.0	0.000	± 0.000	0.000	± 0.000

[1] SE Standard error of n = 3 analytical replicates of lignin extraction from the fraction, each analytical replicate is measured in duplicate at the GC-C-IRMS.
[2] SE Standard error, error propagation calculation included

Table 27 C_4-derived lignin carbon in fractions of the Askov soil.

Fraction		C4-derived lignin (C4-C_{VSC}) concentration / mg g^{-1} fraction				/ mg g^{-1} soil			
		1988		2006		1988		2006	
		Mean	SE[1]	Mean	SE[1]	Mean	SE[2]	Mean	SE[2]
1	bulk soil < 2000 µm	0.0	± 0.0	0.4	± 0.0	0.000	± 0.000	0.377	± 0.037
1L	< 1.85 g cm^{-3}	0.0	± 0.0	8.8	± 1.0	0.000	± 0.000	0.123	± 0.014
1M	1.85 - 2.25 g cm^{-3}	0.0	± 0.0	1.7	± 0.2	0.000	± 0.000	0.058	± 0.007
1H	> 2.25 g cm^{-3}	0.0	± 0.0	0.2	± 0.0	0.000	± 0.000	0.184	± 0.029
2	size fraction 250 - 2000 µm	0.0	± 0.0	0.3	± 0.0	0.000	± 0.000	0.235	± 0.016
2L	< 1.85 g cm^{-3}	0.0	± 0.0	11.6	± 1.0	0.000	± 0.000	0.097	± 0.008
2M	1.85 - 2.25 g cm^{-3}	0.0	± 0.0	2.0	± 0.2	0.000	± 0.000	0.043	± 0.004
2H	> 2.25 g cm^{-3}	0.0	± 0.0	0.2	± 0.0	0.000	± 0.000	0.143	± 0.014
3	size fraction 20 - 250 µm	0.0	± 0.0	0.3	± 0.0	0.000	± 0.000	0.074	± 0.006
3L	< 1.85 g cm^{-3}	0.0	± 0.0	5.4	± 0.3	0.000	± 0.000	0.005	± 0.000
3M	1.85 - 2.25 g cm^{-3}	0.0	± 0.0	1.0	± 0.1	0.000	± 0.000	0.022	± 0.001
3H	> 2.25 g cm^{-3}	0.0	± 0.0	0.1	± 0.0	0.000	± 0.000	0.038	± 0.003
4	size fraction 2 - 20 µm	0.0	± 0.0	0.3	± 0.0	0.000	± 0.000	0.004	± 0.000
5	size fraction < 2 µm	0.0	± 0.0	0.5	± 0.0	0.000	± 0.000	0.000	± 0.000

[1] SE Standard error of n = 3 analytical replicates of lignin extraction from the fraction, each analytical replicate is measured in duplicate at the GC-C-IRMS.
[2] SE Standard error, error propagation calculation included

Table 28 Ratios of lignin monomers as indicators for lignin degradation in fractions.

Fraction		Acid/ aldehyde ratios												Monomer group ratios							
		Ac/Al (V+S)				Ac/Al (V)				Ac/Al (S)				S/V				S/C			
		1988		2006		1988		2006		1988		2006		1988		2006		1988		2006	
		Mean	SE[1]	Mean	SE[1]	Mean	SE[1]	Mean	SE[1]	Mean	SE[1]	Mean	SE[1]	Mean	SE[1]	Mean	SE[1]	Mean	SE[1]	Mean	SE[1]
1	bulk soil < 2000 µm	0.7	± 0.1	0.6	± 0.1	0.6	± 0.1	0.5	± 0.1	0.8	± 0.1	0.6	± 0.1	1.0	± 0.1	1.6	± 0.1	2.9	± 0.2	1.8	± 0.1
1L	< 1.85 g cm^{-3}	0.5	± 0.1	0.3	± 0.0	0.4	± 0.0	0.2	± 0.0	0.6	± 0.2	0.4	± 0.0	0.9	± 0.2	2.0	± 0.1	2.2	± 0.4	1.5	± 0.1
1M	1.85 - 2.25 g cm^{-3}	0.8	± 0.1	0.5	± 0.0	0.7	± 0.1	0.4	± 0.1	0.8	± 0.1	0.6	± 0.0	0.9	± 0.1	1.6	± 0.1	2.8	± 0.2	1.8	± 0.1
1H	> 2.25 g cm^{-3}	0.8	± 0.1	0.6	± 0.0	0.8	± 0.1	0.6	± 0.0	0.8	± 0.1	0.6	± 0.0	0.9	± 0.0	1.5	± 0.0	3.0	± 0.2	1.8	± 0.1
2	size fraction 250 - 2000 µm	0.8	± 0.1	0.5	± 0.1	0.8	± 0.2	0.4	± 0.1	0.8	± 0.0	0.6	± 0.1	0.9	± 0.1	1.7	± 0.2	3.1	± 0.6	1.8	± 0.2
2L	< 1.85 g cm^{-3}	0.4	± 0.0	0.4	± 0.0	0.3	± 0.0	0.2	± 0.0	0.4	± 0.0	0.4	± 0.0	1.1	± 0.0	1.9	± 0.1	2.5	± 0.2	1.6	± 0.2
2M	1.85 - 2.25 g cm^{-3}	0.6	± 0.0	0.5	± 0.0	0.6	± 0.1	0.4	± 0.0	0.7	± 0.1	0.5	± 0.1	1.0	± 0.0	1.6	± 0.1	2.3	± 0.1	1.8	± 0.2
2H	> 2.25 g cm^{-3}	0.9	± 0.1	0.7	± 0.1	0.9	± 0.1	0.6	± 0.1	1.0	± 0.1	0.7	± 0.2	0.9	± 0.1	1.5	± 0.2	3.3	± 0.5	1.8	± 0.2
3	size fraction 20 - 250 µm	0.8	± 0.0	0.6	± 0.0	0.8	± 0.1	0.5	± 0.1	0.8	± 0.0	0.7	± 0.1	1.0	± 0.0	1.5	± 0.1	2.9	± 0.2	1.9	± 0.1
3L	< 1.85 g cm^{-3}	0.4	± 0.0	0.4	± 0.0	0.4	± 0.0	0.3	± 0.0	0.5	± 0.0	0.4	± 0.0	1.0	± 0.0	1.7	± 0.1	2.1	± 0.1	1.6	± 0.1
3M	1.85 - 2.25 g cm^{-3}	0.6	± 0.0	0.5	± 0.0	0.5	± 0.1	0.4	± 0.1	0.6	± 0.1	0.6	± 0.1	1.0	± 0.0	1.5	± 0.1	2.2	± 0.1	1.7	± 0.1
3H	> 2.25 g cm^{-3}	0.9	± 0.1	0.7	± 0.1	0.8	± 0.1	0.6	± 0.2	0.9	± 0.1	0.7	± 0.2	0.9	± 0.1	1.4	± 0.2	3.2	± 0.5	2.3	± 0.2
4	size fraction 2 - 20 µm	0.7	± 0.0	0.5	± 0.0	0.7	± 0.0	0.5	± 0.1	0.7	± 0.0	0.6	± 0.0	0.9	± 0.0	1.4	± 0.1	2.9	± 0.3	2.0	± 0.1
5	size fraction < 2 µm	0.9	± 0.1	0.6	± 0.0	0.9	± 0.1	0.6	± 0.0	0.8	± 0.3	0.5	± 0.0	0.6	± 0.1	1.4	± 0.0	7.1	± 5.2	3.3	± 0.7

[1] SE Standard error of N = 3 analytical replicates of lignin extraction from the fraction, each analytical replicate is measured in triplicate at the GC-FID. Error propagation calculation included.

Scanning electron microscopy (SEM) images

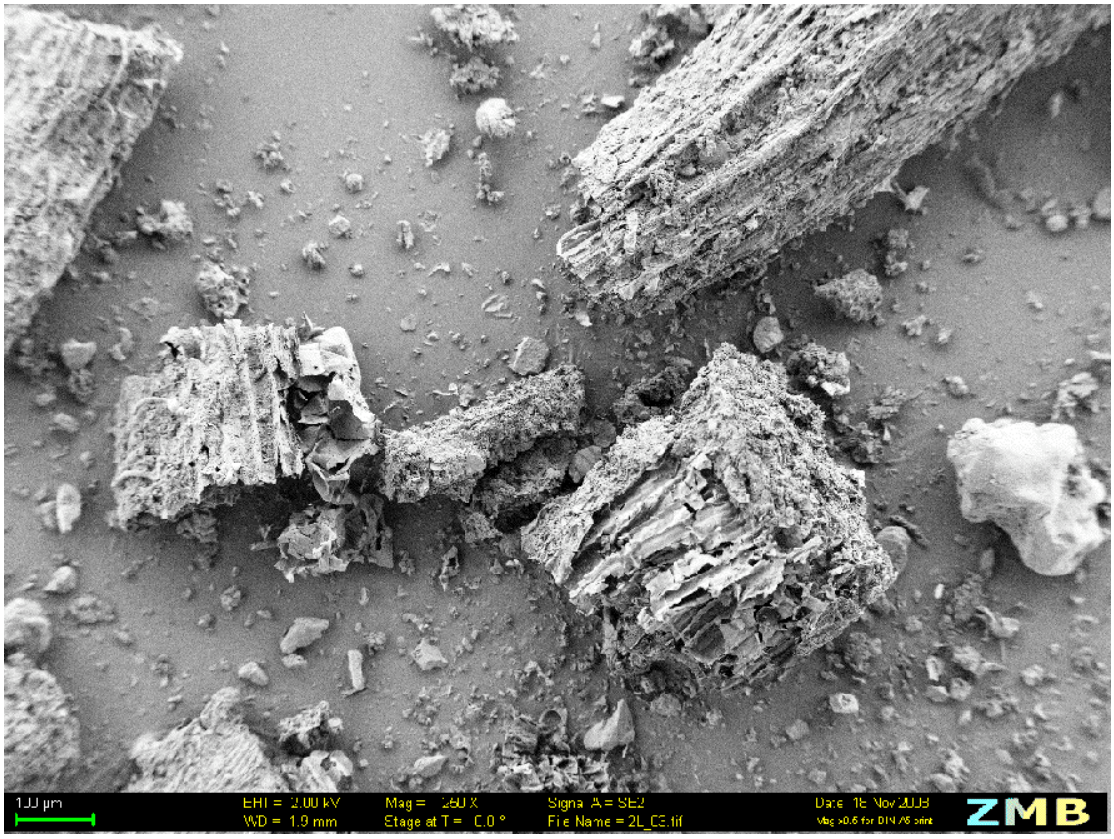

Figure 1 Free particulate organic matter (density < 1.85 g cm^{-3}, size fraction 250-2000 μm)

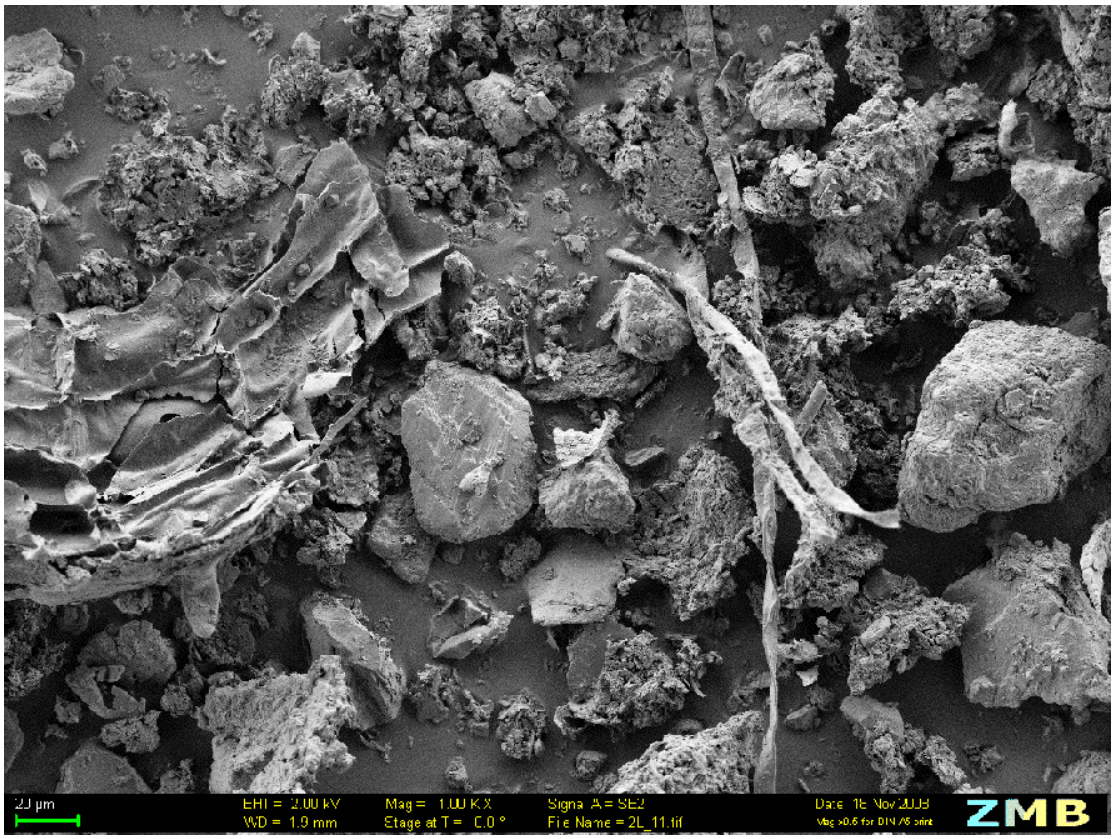

Figure 2 Free particulate organic matter (density < 1.85 g cm^{-3}, size fraction 250-2000 μm)

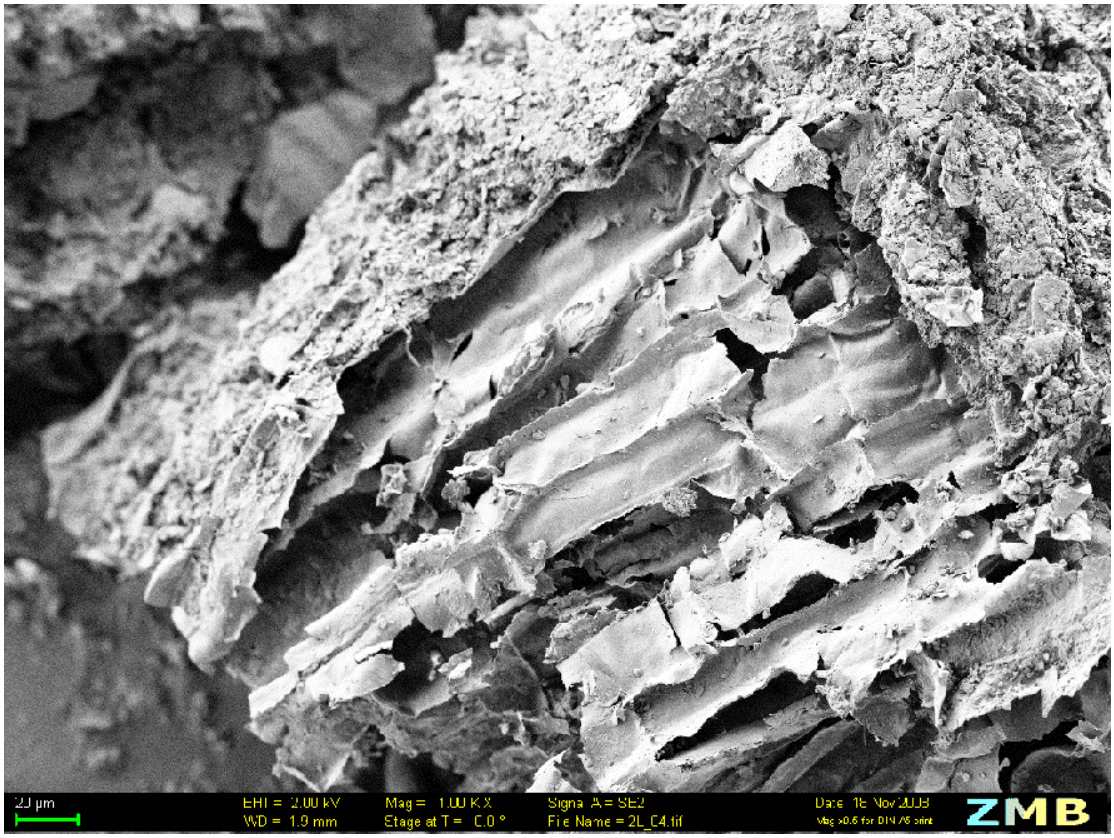

Figure 3 Free particulate organic matter (density < 1.85 g cm^{-3}, size fraction 250-2000 μm)

Figure 4 Free particulate organic matter (density < 1.85 g cm^{-3}, size fraction 250-2000 μm)

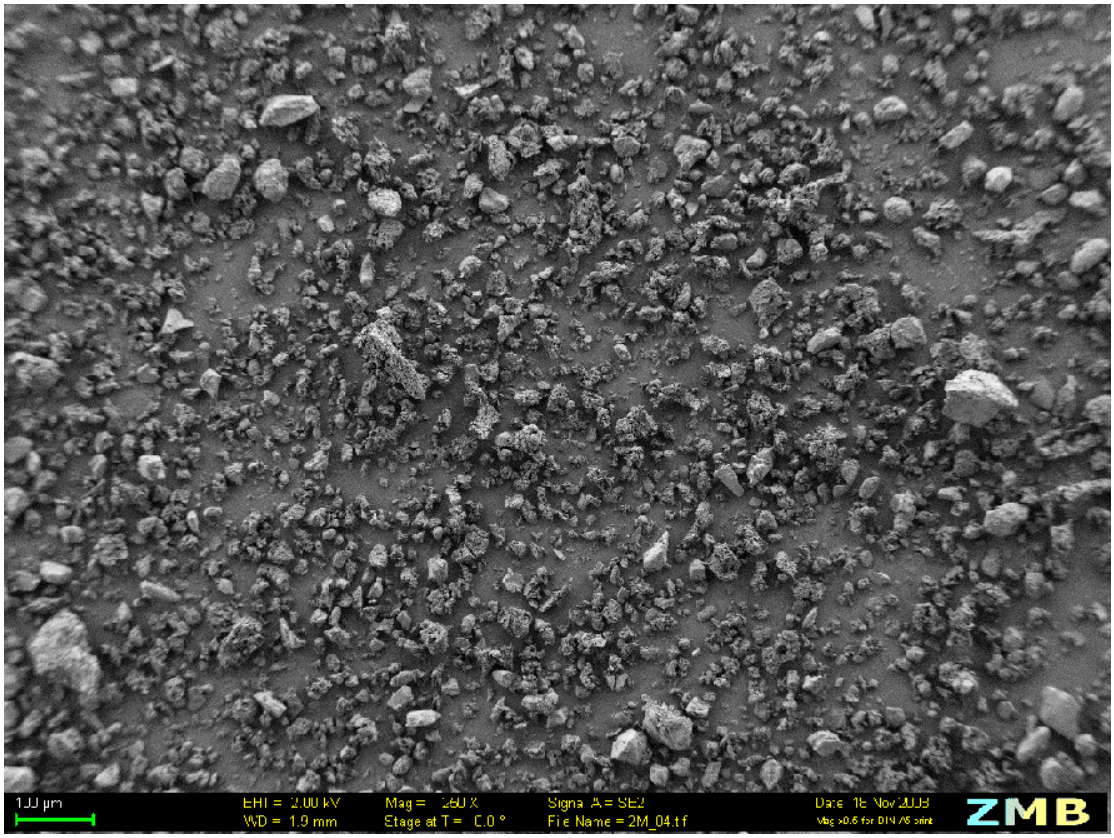

Figure 5 Aggregates (density 1.85-2.25 g cm^{-3}, size fraction 250-2000 μm)

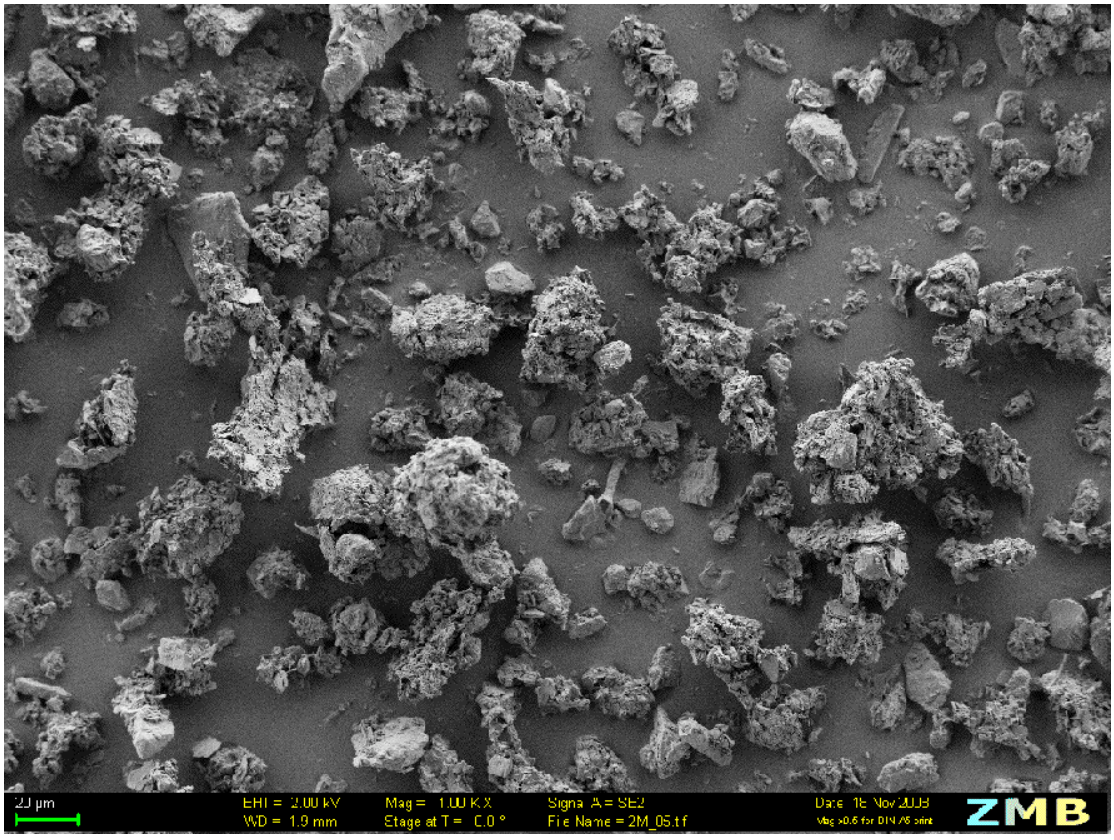

Figure 6 Aggregates (density 1.85-2.25 g cm^{-3}, size fraction 250-2000 μm)

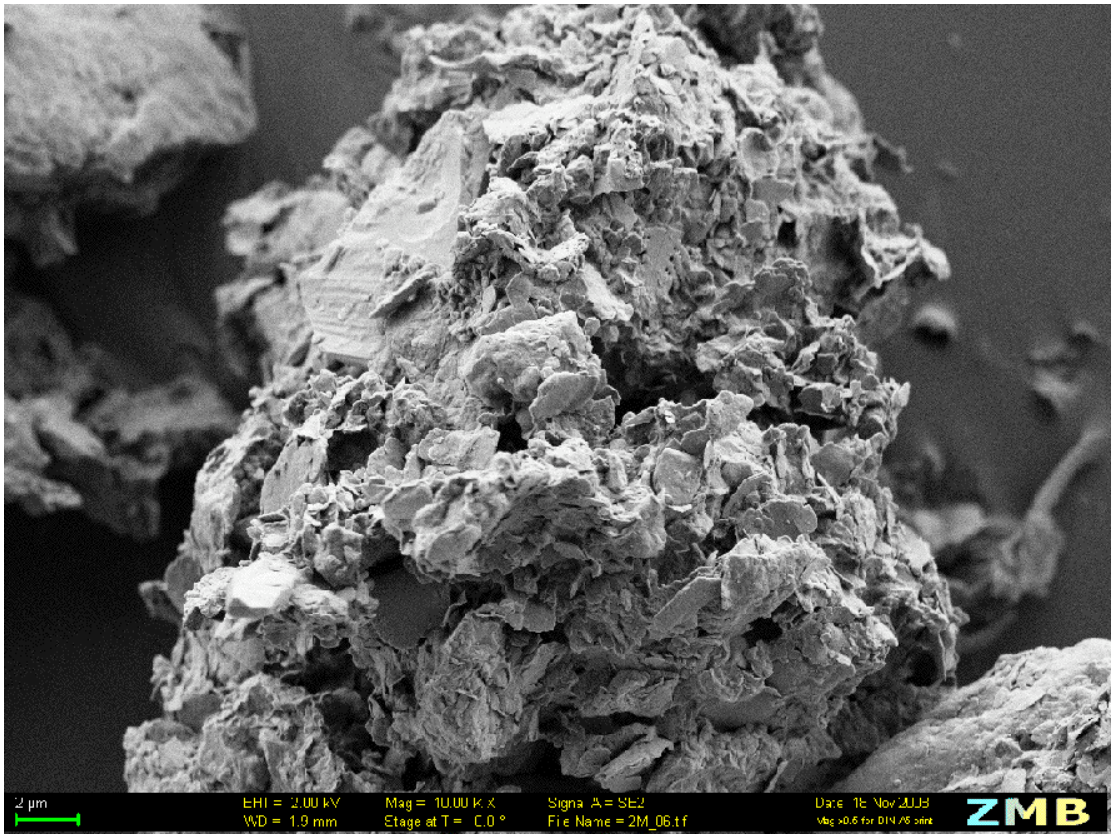

Figure 7 Aggregate close-up (density 1.85-2.25 g cm^{-3}, size fraction 250-2000 μm)

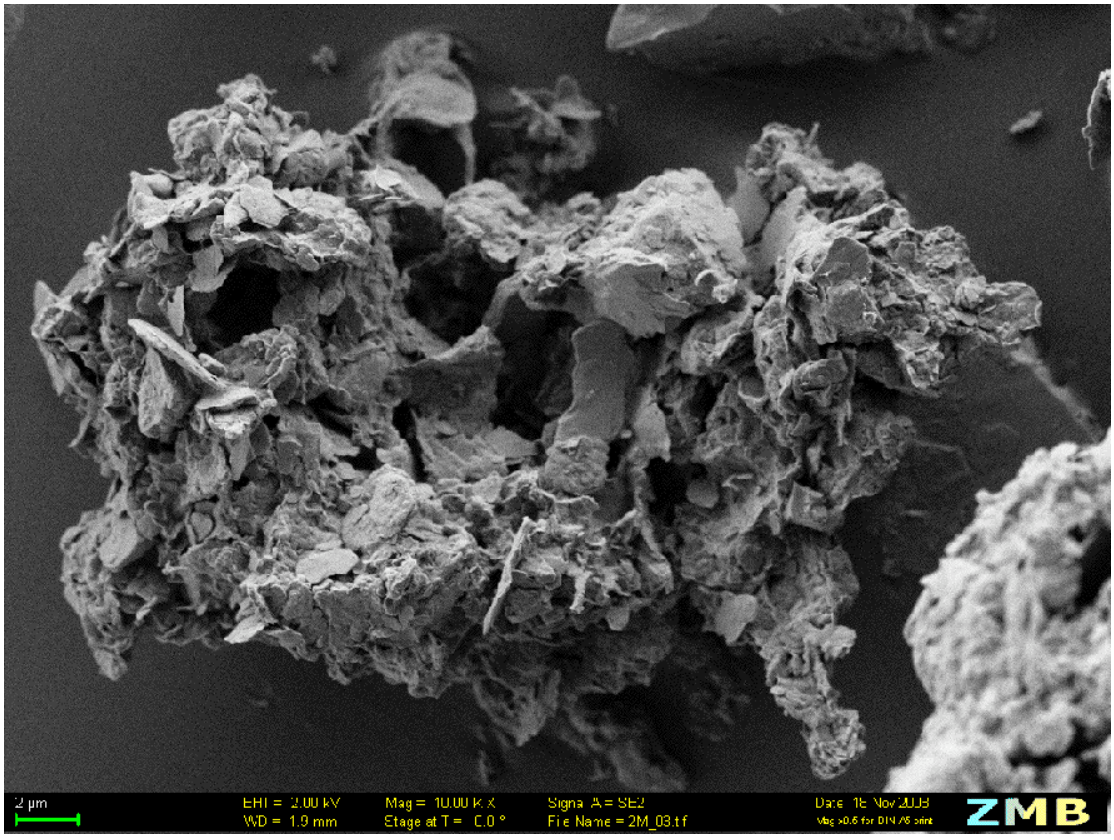

Figure 8 Aggregate close-up (density 1.85-2.25 g cm^{-3}, size fraction 250-2000 μm)

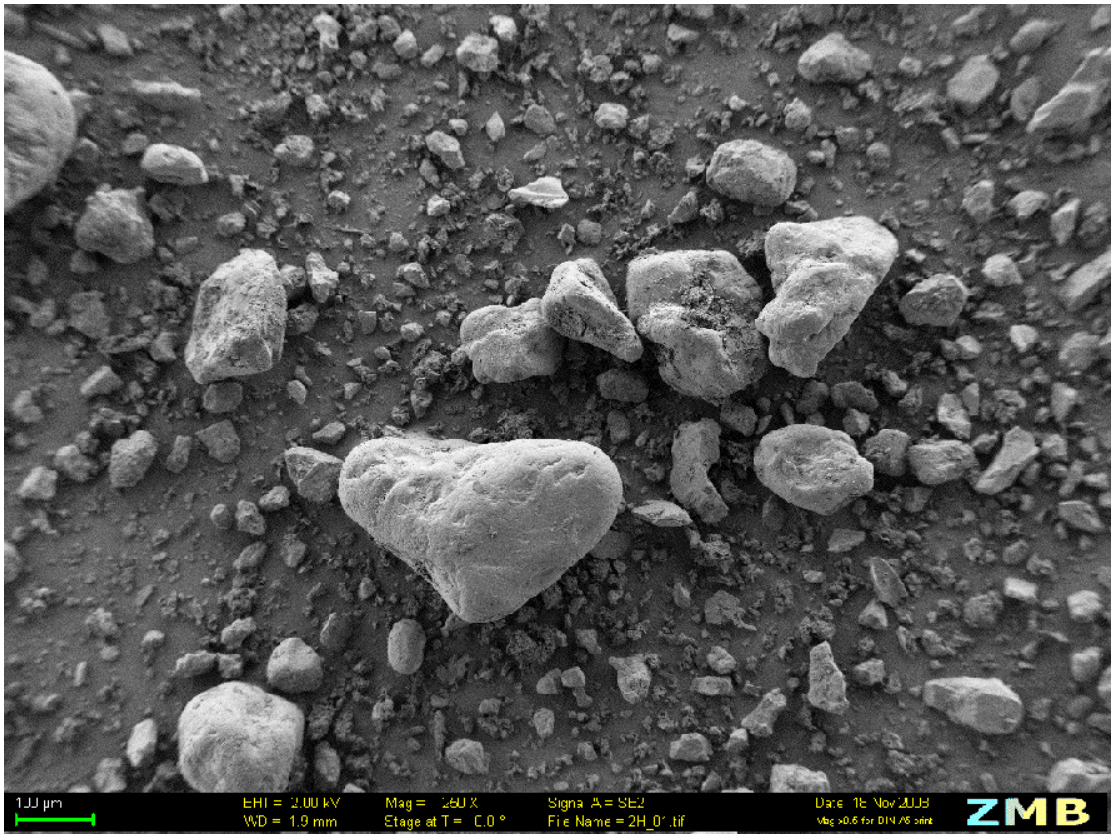

Figure 9 Mineral particles (density > 2.25 g cm^{-3}, size fraction 250-2000 μm)

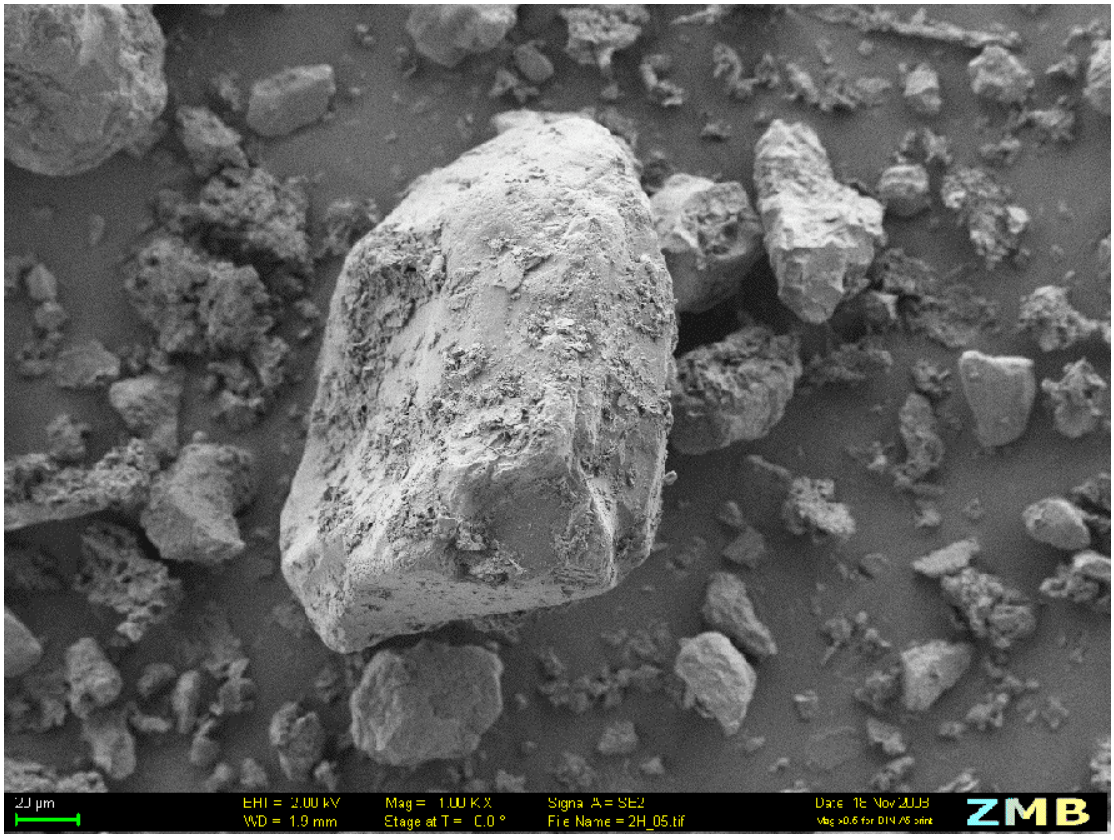

Figure 10 Mineral particles (density > 2.25 g cm^{-3}, size fraction 250-2000 μm)

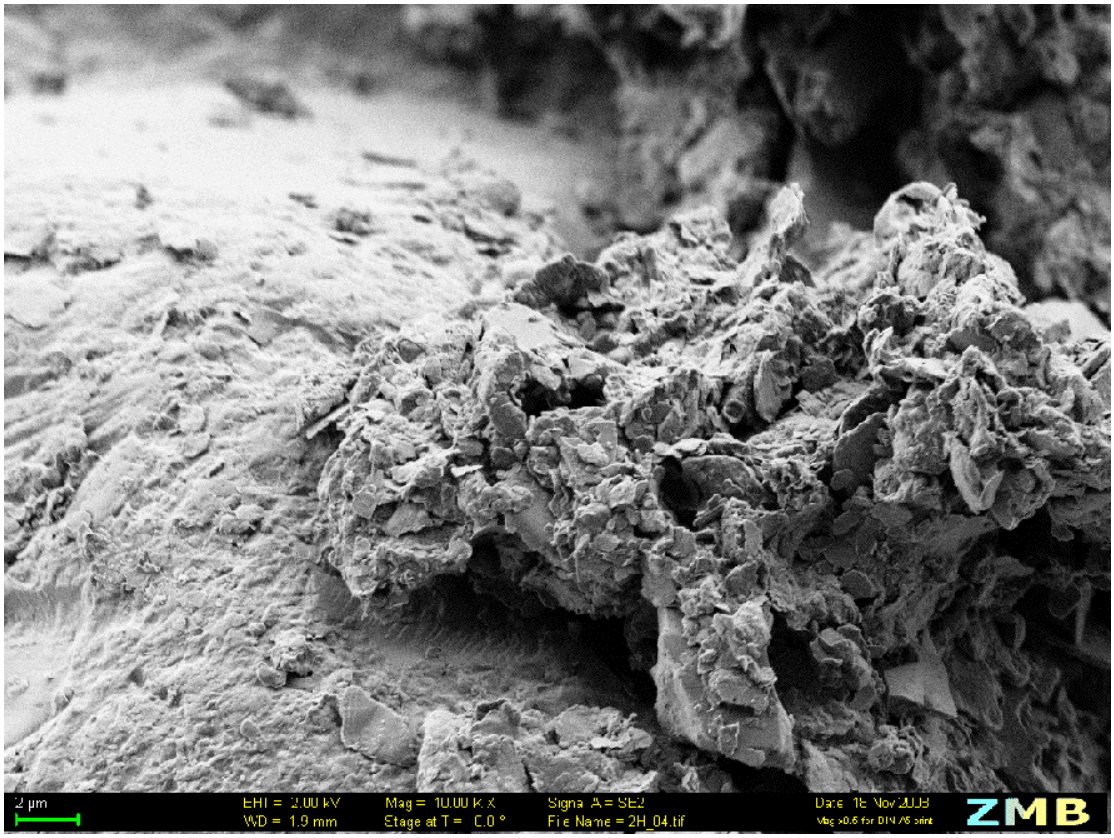

Figure 11 Aggregate on surface of mineral particle (density > 2.25 g cm^{-3}, size fraction 250-2000 μm)

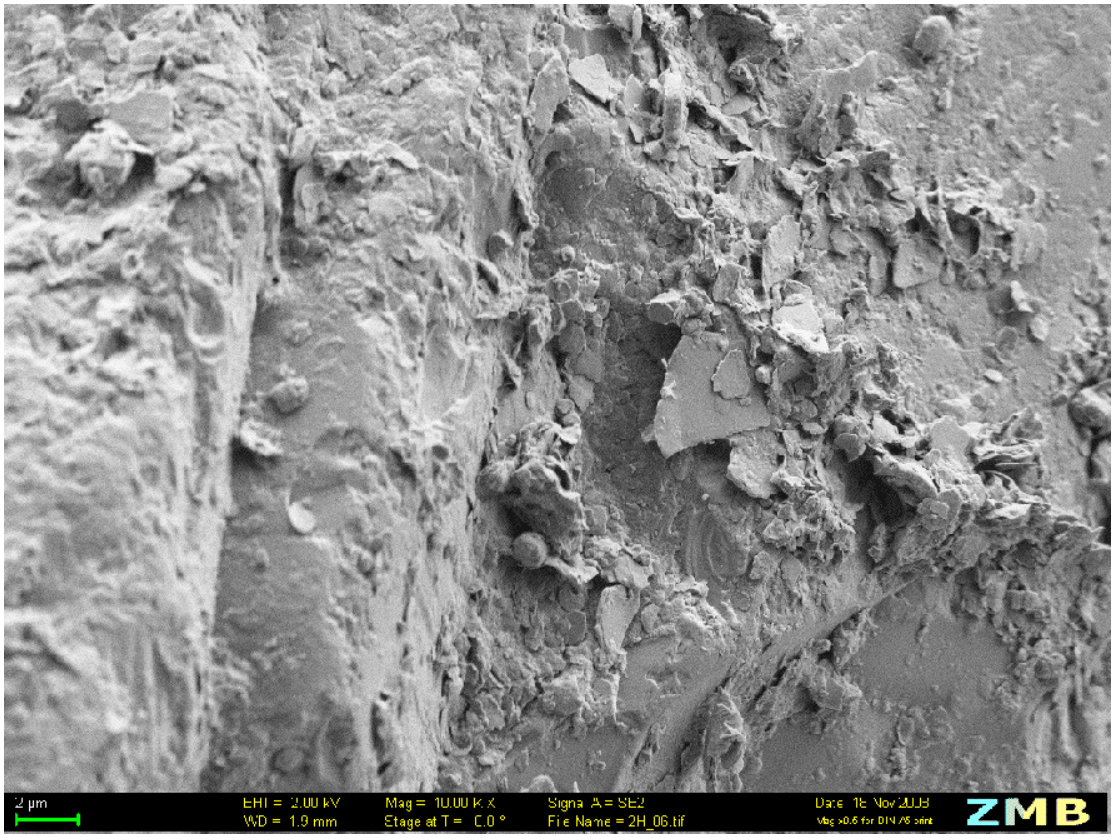

Figure 12 Surface of mineral particle (density > 2.25 g cm^{-3}, size fraction 250-2000 μm)

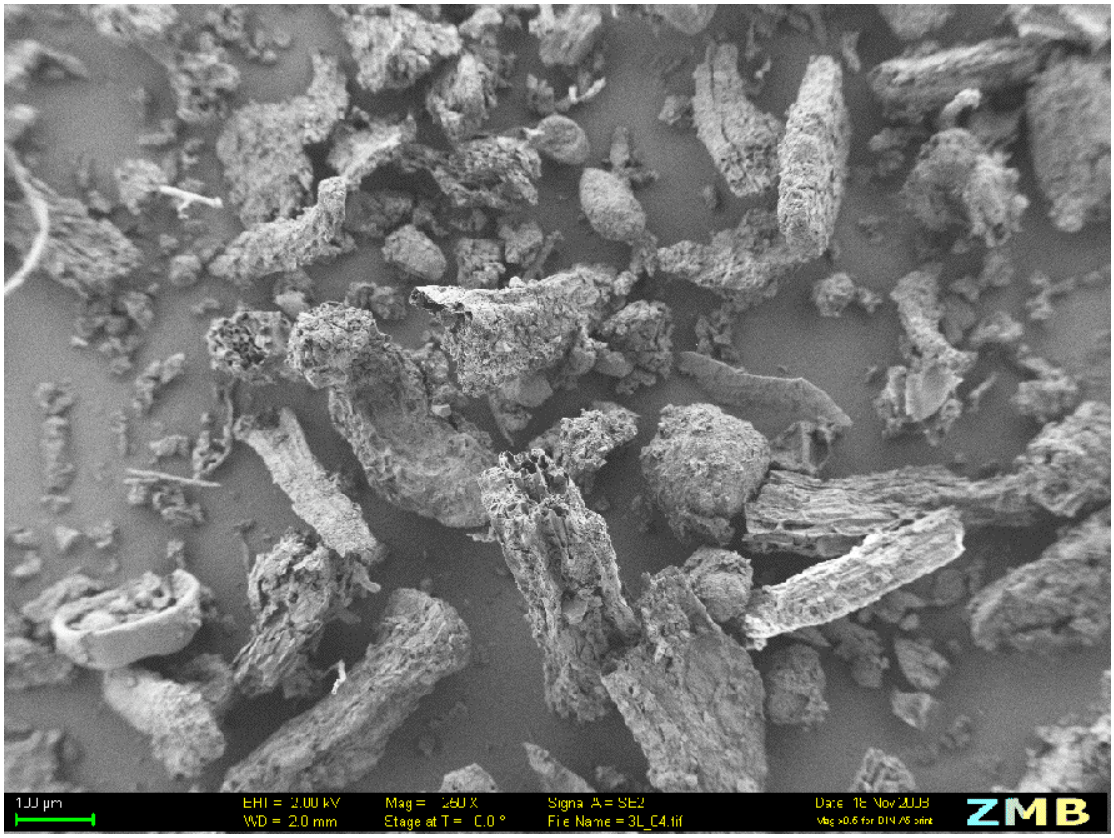

Figure 13 Free particulate organic matter (density < 1.85 g cm^{-3}, size fraction 20-250 μm)

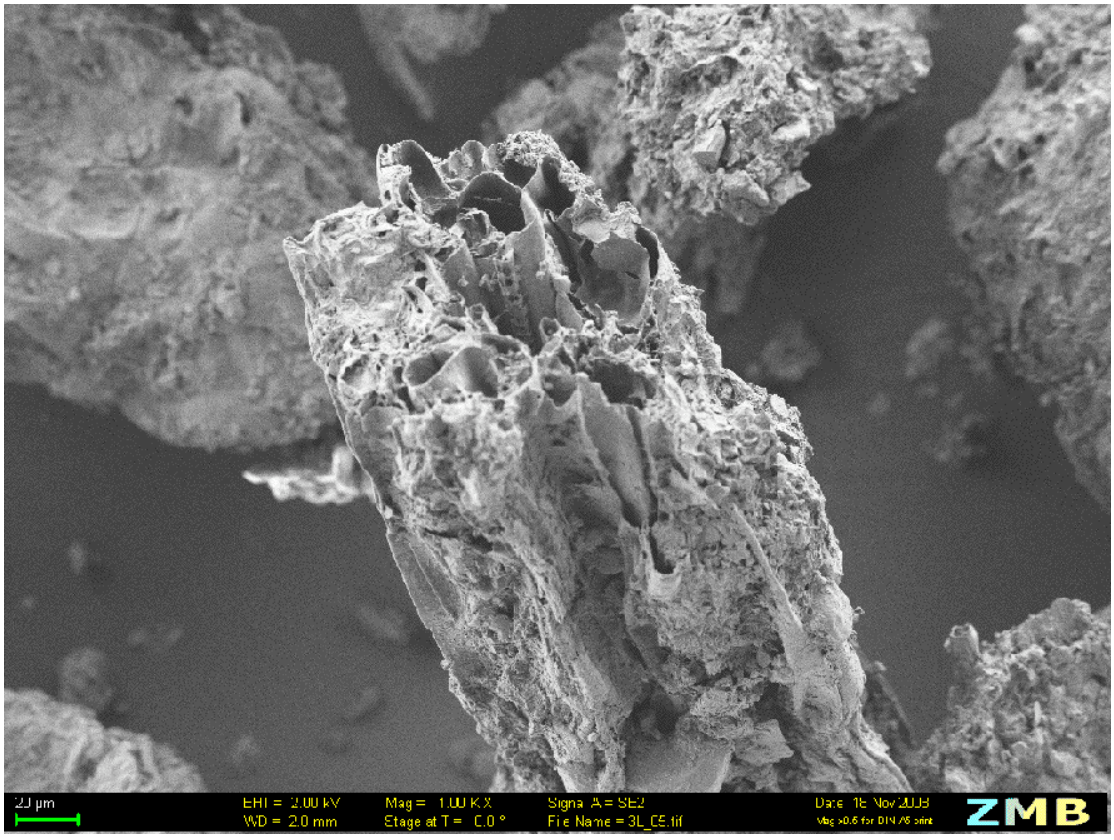

Figure 14 Free particulate organic matter (density < 1.85 g cm^{-3}, size fraction 20-250 μm)

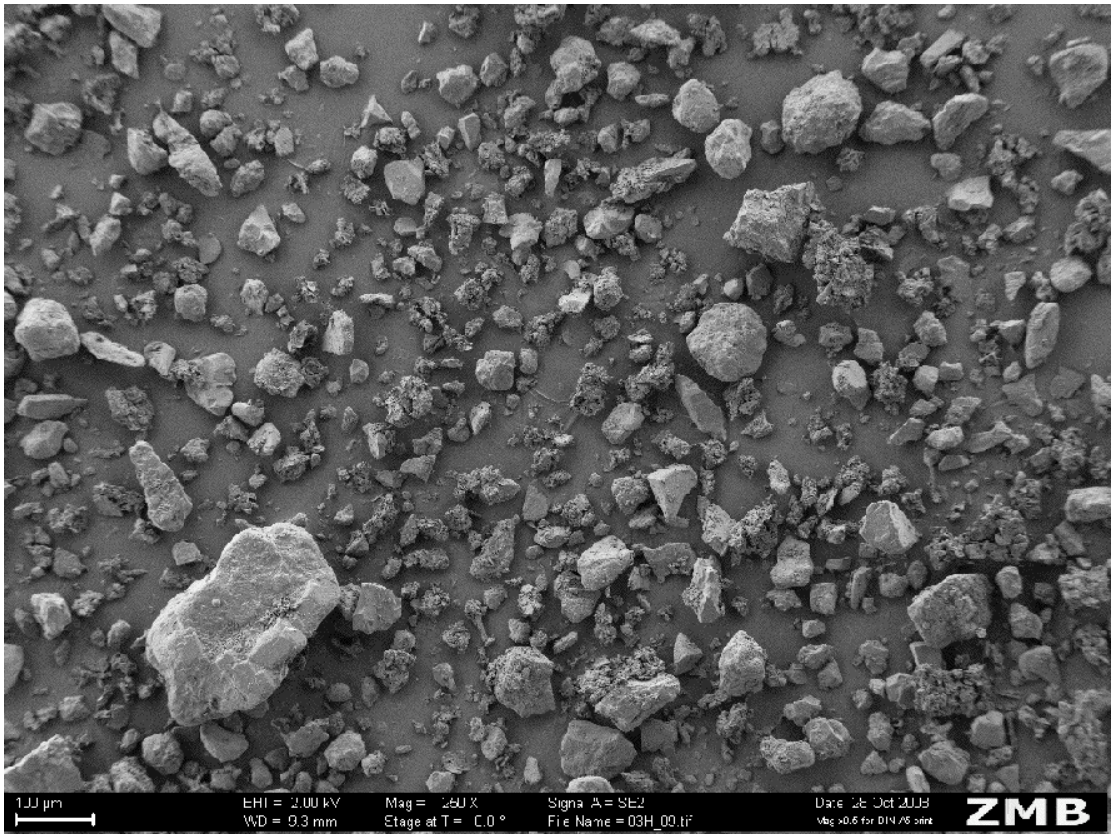

Figure 15 Mineral particles and aggregates (density 1.85-2.25 g cm^{-3}, size fraction 20-250 μm)

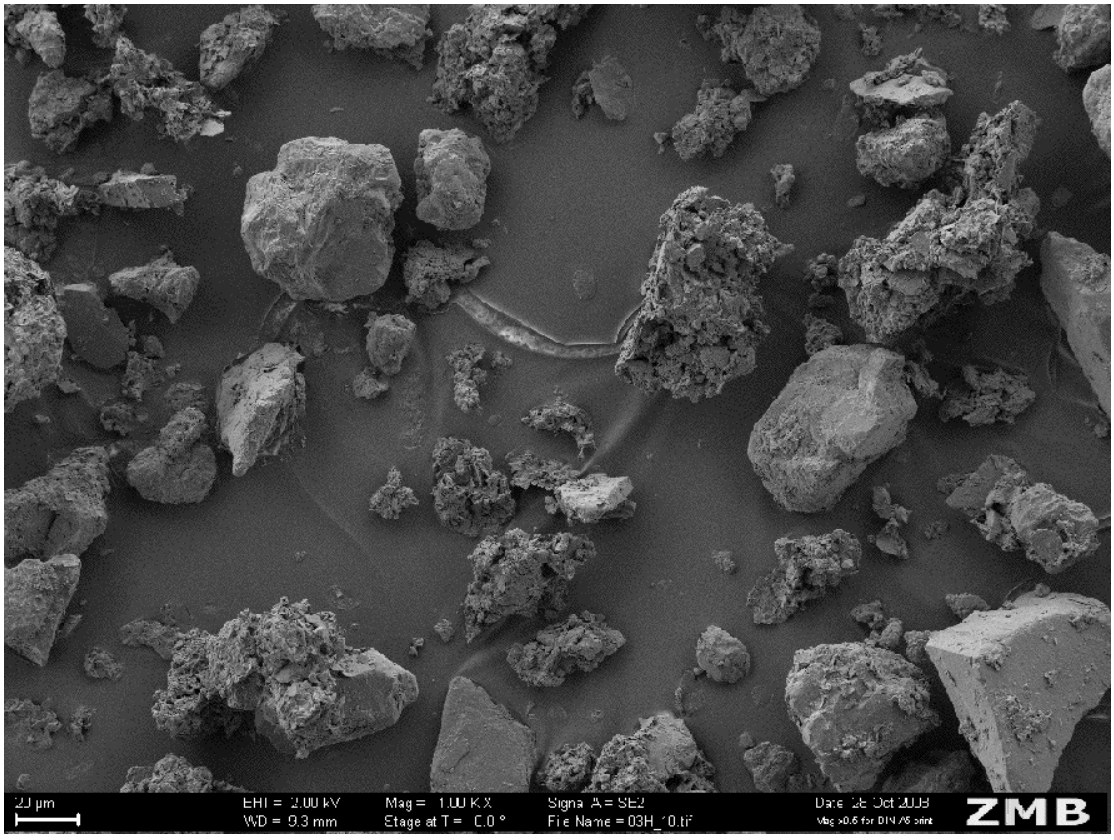

Figure 16 Mineral particles and aggregates (density 1.85-2.25 g cm^{-3}, size fraction 20-250 μm)

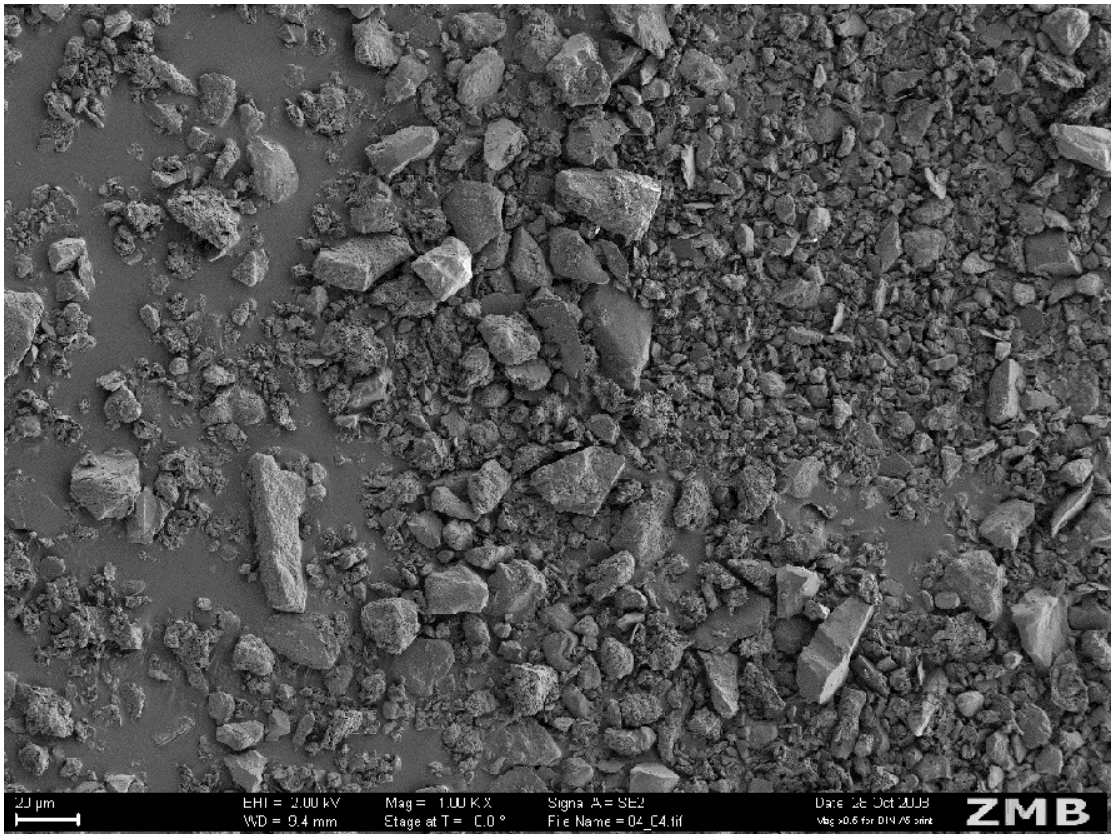

Figure 17 Mineral particles and aggregates (size fraction 2-20 μm)

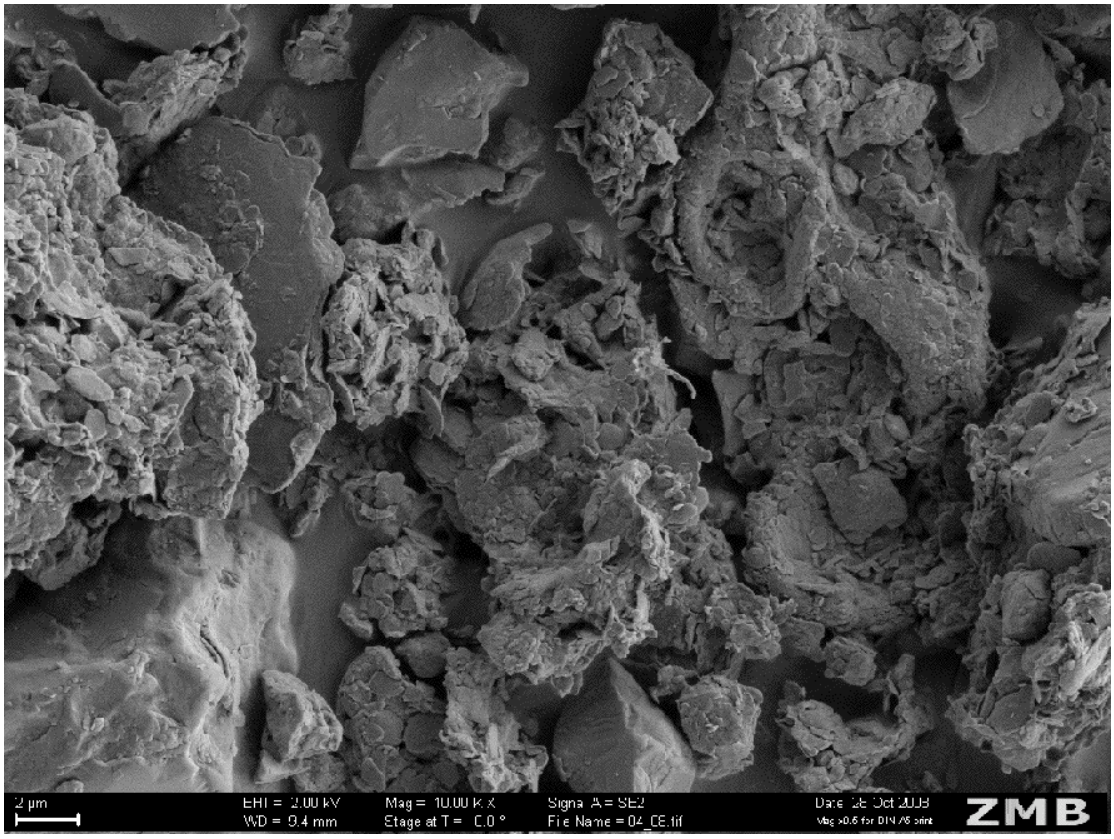

Figure 18 Close-up of silt-sized mineral particles and aggregates (size fraction 2-20 μm)

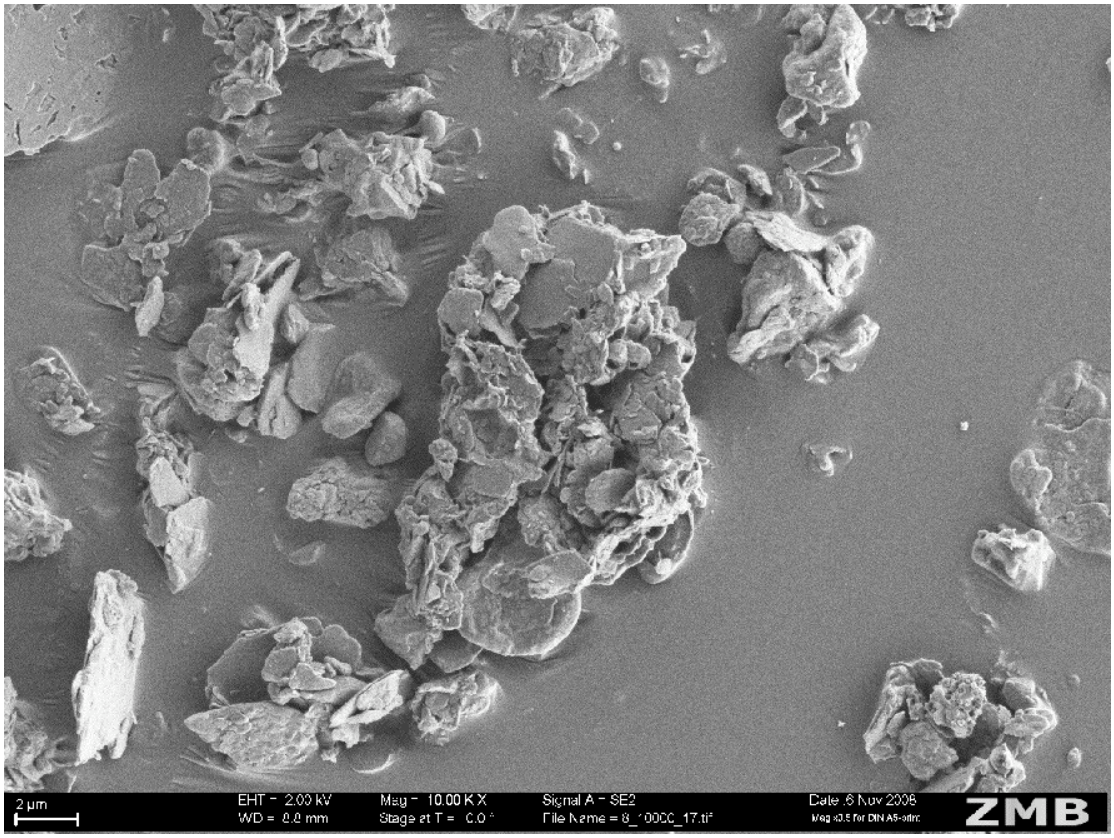

Figure 19 Clay-sized aggregate (size fraction < 2 μm)

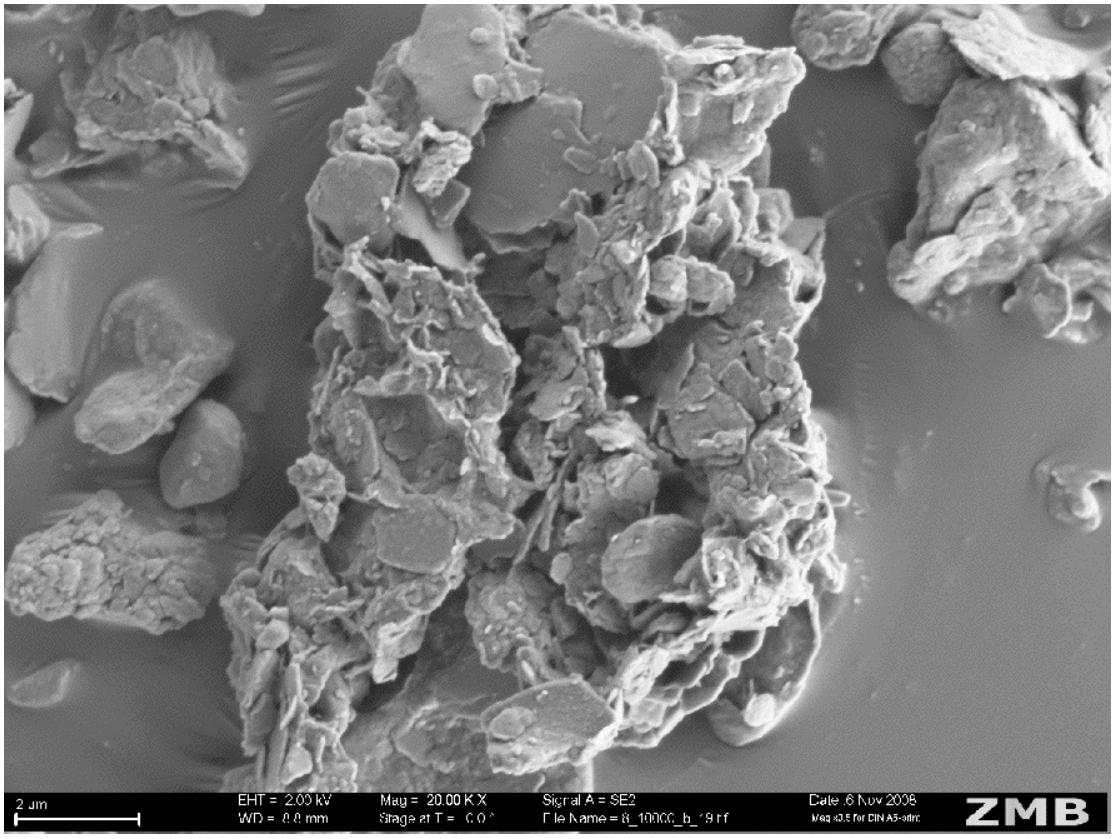

Figure 20 Close-up of clay-sized aggregate (size fraction < 2 μm)

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Curriculum vitae

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Education

Aug. 2005 - Sept. 2009	Dissertation, University of Zurich (Switzerland), Department of Geography, Physical Geography, Soil Science & Biogeography
Oct. 1999 - July 2005	Diploma in Agricultural Sciences, Martin-Luther-University Halle-Wittenberg (Germany), Institute of Agricultural and Nutritional Sciences, fields of study: Soil science, Land-use planning, Organic farming, Pomiculture
July 1999	Abitur, Landesschule Pforta, Schulpforte (Germany)

Academic positions and research experience

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Jan. 2002 - March 2004	Undergraduate research assistant, Soil Chemistry Lab, Martin-Luther-University Halle-Wittenberg, Institute of Agricultural and Nutritional Sciences

Professional Training

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Workshop “Application of isotopes in pedological process-related studies”, University of Hohenheim, Department of Soil Science, Stuttgart-Hohenheim, Germany, 08. - 12. Feb. 2006

Workshop “Stable isotopes in ecology”, Paul Scherrer Institute, Villigen, Switzerland, 17. - 20. Jan. 2006

Memberships

Deutsche Bodenkundliche Gesellschaft DBG (German Soil Science Society),
Working Group Soil in Education and Society

Contributions at international conferences

oral presentations:

EUROSOIL 2008, Vienna, Austria, August 2008

Nordic Association of Agricultural Scientists, Sandbjerg, Denmark, June 2008

European Geosciences Union (EGU) General Assembly, Vienna, Austria, April 2008

German Soil Science Society Biannual Meeting, Dresden, Germany, September 2007

International Symposium on Organic Matter Dynamics in Agro-Ecosystems, Poitiers, France, July 2007

poster presentations:

13th Meeting of the International Humic Substances Society (IHSS), Karlsruhe, Germany, August 2006

18th World Congress of Soil Science, Philadelphia, USA, July 2006

Publications diploma thesis

Hofmann, A., Wittenmayer, L., Arnold, G., Schieber, A., Merbach, W. (2009): Root exudation of phloridzin by apple seedlings (*Malus* x *domestica* Borkh.) with symptoms of apple replant disease. Journal of Applied Botany and Food Quality 82, 193-198.

Hofmann, Anett: Die Symptomausprägung der Specific Apple Replant Disease (SARD) bei Apfelsämlingen (*Malus* x *domestica* Borkh. 'Bittenfelder Sämling') und deren Beziehung zum phenolischen Wurzelexudate Phloretin-2'-*O*-b-D-glucosid. Beiträge aus der Hallenser Pflanzenernährungsforschung Bd. 9. Verlag Grauer, Beuren, Stuttgart, 2005.

We shall not cease from exploration
And the end of all our exploring
Will be to arrive where we started
And know the place for the first time.

T.S. Eliot, Four Quartets